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SUPPLEMENTARY MATERIAL

Selective and Stable DNA Base Pairing Without Hydrogen Bonds

Tracy J. Matray, Eric T. Kool*, Department of Chemistry, University of Rochester, Rochester, NY 14627

Oligodeoxynucleotide synthesis. 5'-*O*-tritylated phosphoramidite analogues of nucleosides **1** and **2** were synthesized as previously reported.[1,2] Oligonucleotides were synthesized on an Applied Biosystems 392 synthesizer using standard β-cyanoethyl phosphoramidite chemistry, but with extended (10 min) coupling cycles for the modified nucleotides. Stepwise coupling yields for the nonnatural residues were all greater than 93% as determined by trityl cation response. All oligomers were deprotected in concentrated NH_3 (55 °C, 12 h), purified by preparative 20% denaturing polyacrylamide gel electrophoresis, and isolated by excision and elution from the gel. The recovered material was subsequently quantitated by absorbance at 260 nm with molar extinction coefficients determined by the nearest neighbor method.[3] Values for oligonucleotides containing **1** were estimated by measuring the absorbance at 350 nm and subtracting 0.48 of this value from the total absorbance at 260 nm to get the absorbance of the natural DNA alone, as described.[4] Intact incorporation of nucleotide **1** was confirmed by electrospray mass spectroscopy of an oligonucleotide containing a single pyrene residue (calc. mass: 8620.87; found: 8620.48).

Thermal denaturation studies. Solutions for the thermal denaturation studies contained a 1:1 ratio of two complementary oligomers. Duplex concentrations were 0.5 to 10 μM. The buffer contained NaCl (100 mM), $MgCl_2$ (10 mM), and Na•PIPES (10 mM), pH 7.0. After the solutions were prepared they were heated to 90 °C for 5 min and allowed to cool to room temperature for at least 1 h prior to the melting experiments. Melting studies were carried out in Teflon-stoppered 1 cm path length quartz cells under a nitrogen atmosphere on a Varian Cary 1 UV-vis spectrophotometer equipped with a thermoprogrammer. Absorbance was monitored while temperature was raised from 5 to 80 °C (90 °C for duplexes containing multiple P-ϕ pairs) at a rate of 0.5 °C/min. All sequences were monitored at 260 nm. In most cases (see text) the complexes displayed sharp, apparently two state transitions, with all-or-none melting from bound duplex to free oligomers (see Fig. 1 (suppl.). Computer fitting of the melting data provided both melting temperatures (T_m) and free energy values for the complexes. The free energies were also calculated from van't Hoff plots by plotting $1/T_m$ versus $\ln(C_T/4)$ (see Fig. 2 (suppl.); in both cases close agreement was observed, indicating that the two state approximation is not unreasonable for these specific sequences.

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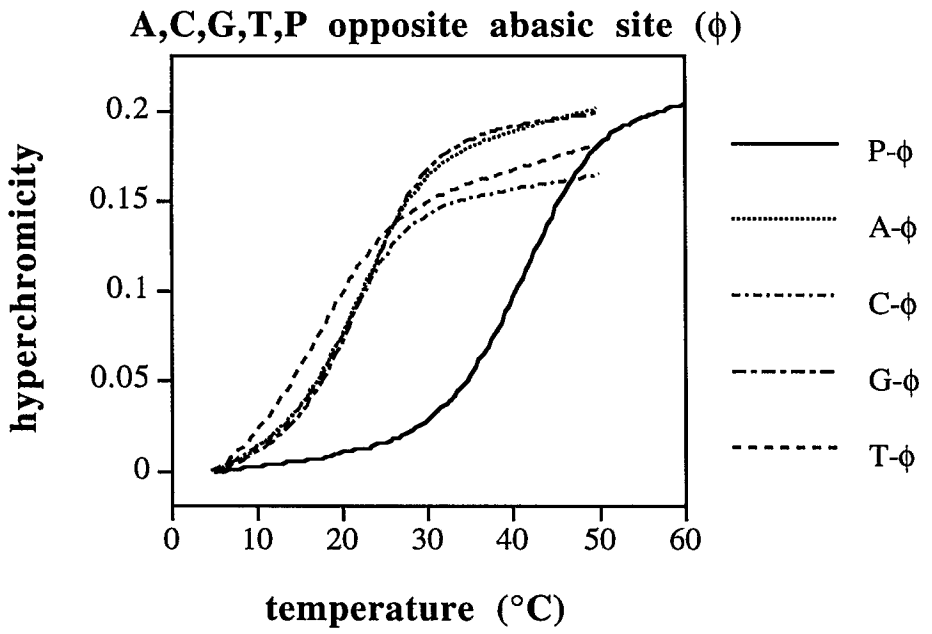

Fig. 1 (suppl.). Sample denaturation curves measured for five different duplexes in this study. The data were monitored at 260 nm.

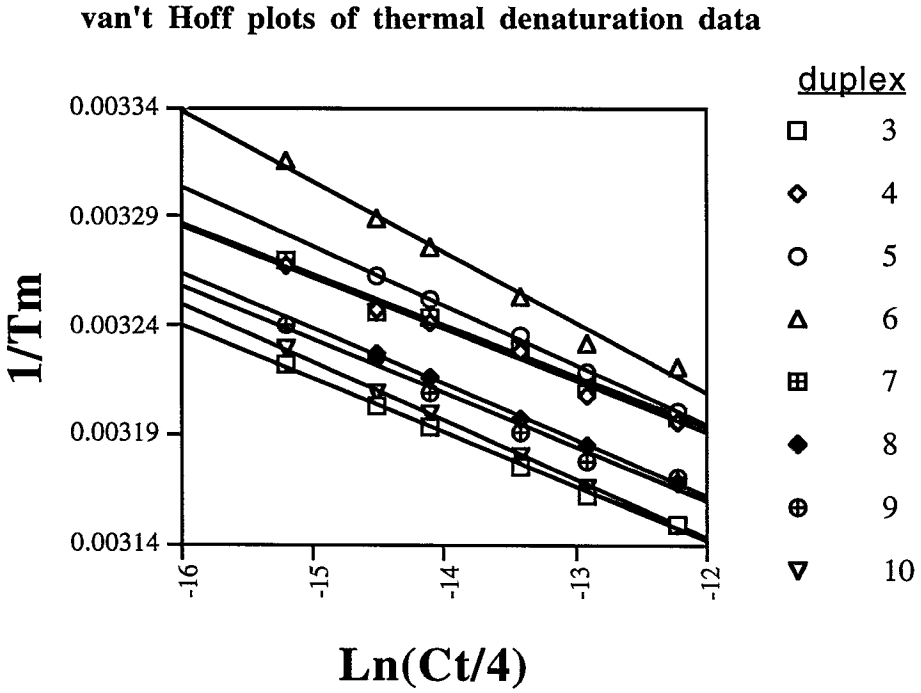

Fig. 2 (suppl.). van't Hoff plots for eight different duplexes in this study. Slope is R / $\Delta H°$, and y-intercept is $\Delta S° / \Delta H°$. Least squares fit values (R^2) are 0.98 or greater.

Determination of binding stoichiometry. Solutions of complementary oligonucleotides were prepared at 10.0 μM total oligomer concentration, with NaCl (100 mM), $MgCl_2$ (10 mM), and Na•PIPES (10 mM) at pH 7.0. These solutions were prepared in various mole ratios (0.0, 0.1, 0.2, 0.3...1.0) of the two oligomers so that final concentrations remained constant at a total of 10.0 μM. To each of the solutions was added a portion of 5'-end-labeled oligonucleotide.[4] The solutions (20 μL) were heated to 90 °C for 5 min and then allowed to cool slowly to room temperature over at least 1 h. Loading buffer ((5 μL), 30% glycerol, 0.25% bromophenol blue, 0.25% xylene cyanol) was added before equal aliquots were loaded onto a 20% nondenaturing polyacrylamide gel. Phosphorimaging of the resulting gel shift assay, enabled quantification of integrated volumes of bands representing both single stranded and duplex material. Using these volumes, percentage of uncomplexed single-stranded oligonucleotide was calculated for each lane.

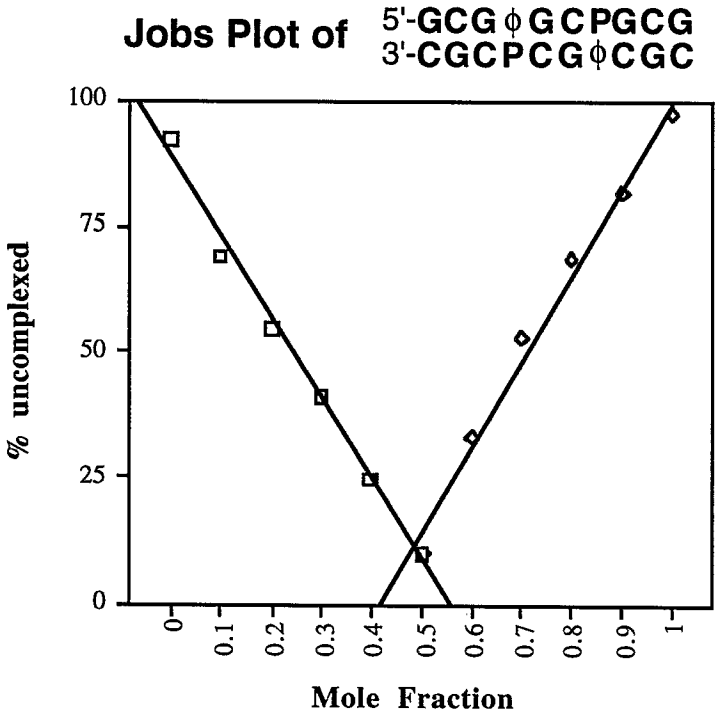

Fig. 3 (suppl.). Jobs plot of percent uncomplexed strands (measured by phosphorimaging of nondenaturing gel) vs. mole ratio strand A (5'-dGCGϕGCPGCG) to strand B (5'-dCGCϕGCPCGC), showing intersection at 0.48 mole ratio, indicating a 1:1 complex is formed.

CD spectroscopy. Circular dichroism spectra were measured using a Jasco J-710 spectropolarimeter (Jasco Co., Tokyo) between 320 and 200 nm in the standard buffer containing NaCl (100 mM), $MgCl_2$ (10 mM), Na•PIPES (10 mM) at pH 7.0. Single stranded or duplex oligonucleotides were at a concentration of 5 μM, and spectra were taken at variable temperatures (5, 20, 40, 60, and 75 °C). For each spectrum, data points were taken every 1 nm using a bandwidth setting of 1 nm at a speed of 50 nm/min. Individual spectra were smoothed using the program provided by the manufacturer.

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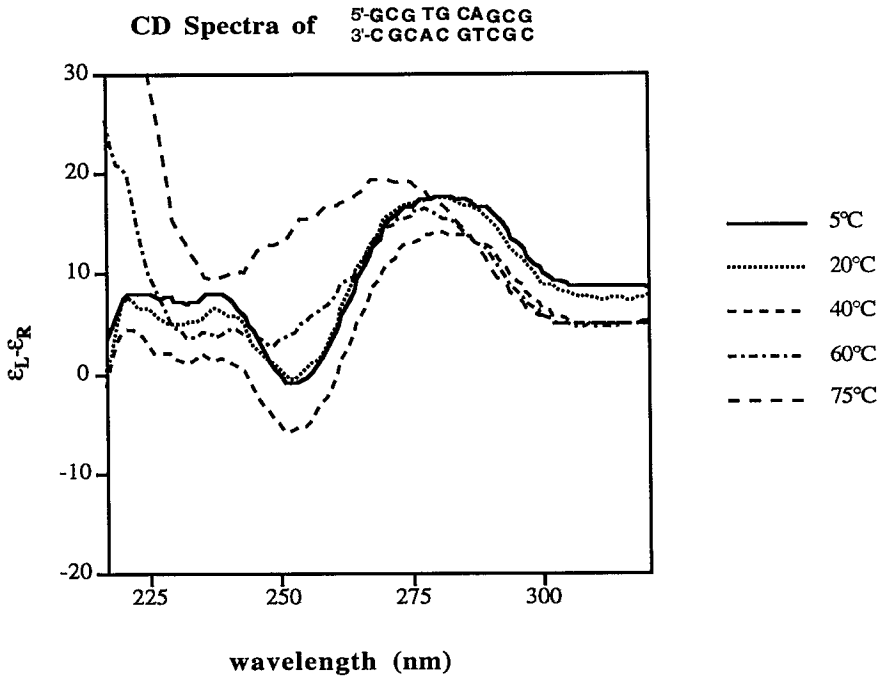
A



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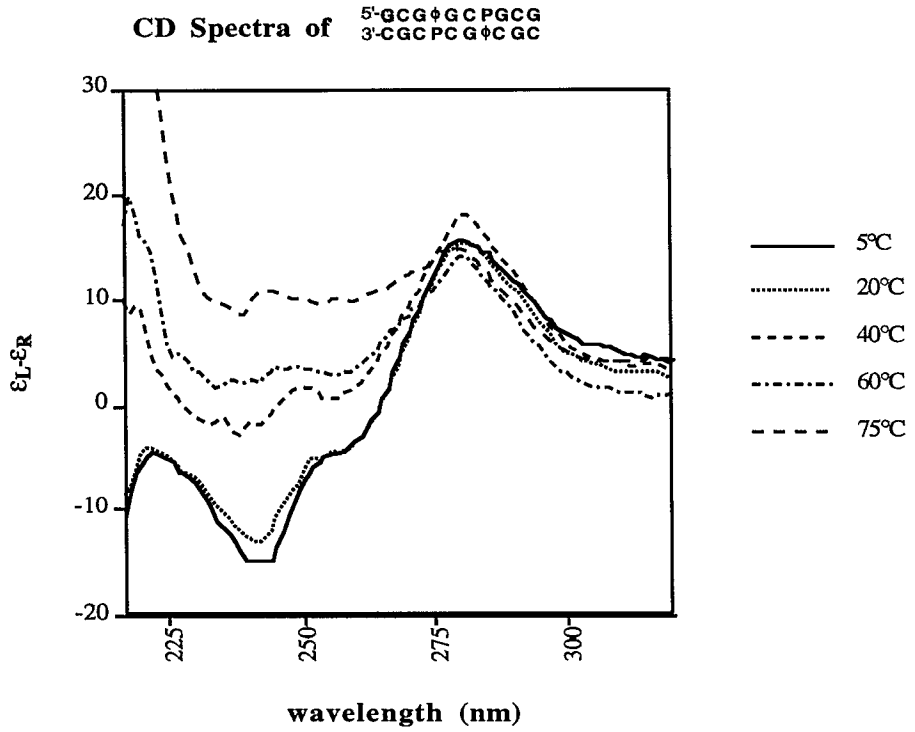

Fig. 4 (suppl.). Temperature dependence of CD spectra of control duplex **16** containing 20% A-T pairs (A) and duplex **17** containing 20% P-ϕ pairs (B). Duplex concentration is 5 μM.

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References for supplementary material

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