Use of Hydrophobic Substituents in Controlling Self-Assembly of Oligonucleotides

R. L. Letsinger,* S. K. Chaturvedi, F. Farooqui, and M. Salunkhe

Department of Chemistry
Northwestern University
Evanston, Illinois 60208

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The base pairing motifs of oligonucleotides serve as simple but effective guides for constructing self-assembling molecular systems; however, the types of structures attainable from unmodified oligonucleotides are relatively limited. To increase the range and stability of assemblies derived from oligonucleotides, we and others have previously explored the effects of introducing non-nucleotide structural elements such as tethered intercalating groups,[1] cationic substituents,[2] and aliphatic and aromatic bridges.[3,4] We report here that appropriate positioned hydrophobic substituents can serve as powerful aids in organizing oligonucleotide blocks in dilute aqueous media. Examples are provided demonstrating synergism for hydrophobic and base-pairing/base-stacking interactions in stabilizing both duplex and triplex oligonucleotide structures.

Cholesteryl was selected as a modifying substituent since cholesteryl oligonucleotide conjugates can be readily prepared[4] and the hydrophobic effect should be significant. In addition, a number of recent papers have reported interesting biological activity for cholesteryl-modified oligonucleotides.[4,5]

Comparative T_m values for dissociation of several complexes of oligonucleotides containing cholesteryl groups are presented in Table I.[6] In these compounds, cholesteryl is tethered to phosphorus at a 5′ or 3′ terminal internucleoside link *via* an oxycarbonylaminoethylamino group[4a] (*ch*) or to a 5′ terminal oxygen atom by a phosphodiester link[4b] (*ch′*). As previously noted,[4a] a cholesteryl substituent near the end of one strand has little effect on the stability of a duplex (compare expts 1,2 and 4,5). On the other hand, when each oligomer has a cholesteryl group tethered near the terminus and the hydrophobic groups are favorably positioned to overlap and aggregate when the strands hybridize, a large increase in stability of the duplex is observed

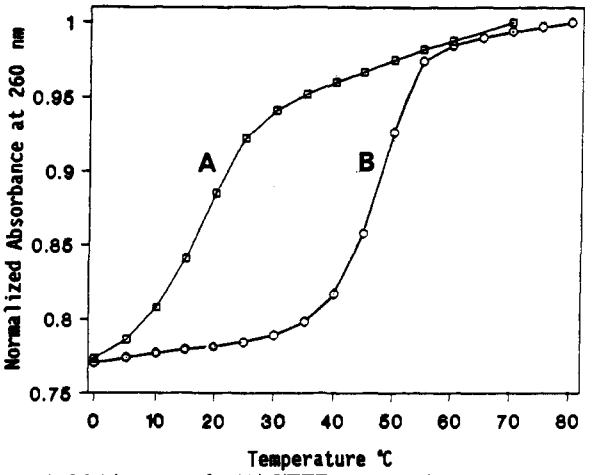

Figure 1. Melting curves for (A) 5′TTTTTTTTT*ch*T + AAAAAAAAA and (B) 5′TTTTTTTTT*ch*T + 3′AAAAAAAAA*ch′*, in 0.10 M NaCl, 10 mM Tris·HCl buffer, pH 7.0. The concentration of each oligomer was 5 μM.

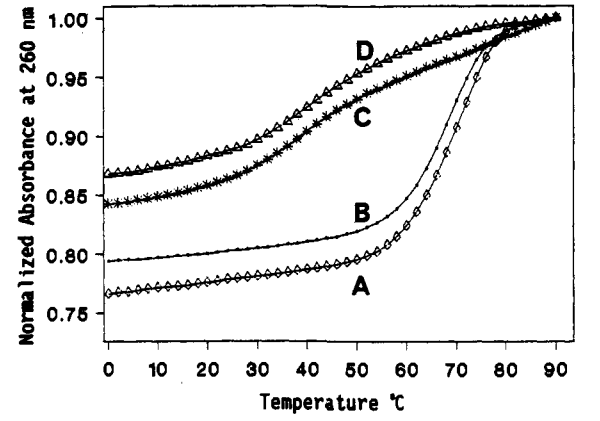

Figure 2. Melting curves for mixed-base decamer pairs differing in cholesteryl positions. Curves A, B, C, D correspond to expts 10, 11, 12, 13 in Table I, respectively.

(Figures 1 and 2 and Table I; compare expts 3 with 1,2, 6 with 4,5, 8 with 7, and 10,11 with 9). The increase of 23°–34° in T_m for the doubly substituted derivative relative to the unsubstituted duplex resembles the increase found for palindromic oligonucleotides linked by a covalent bridge.[3] It seems likely that the cholesteryl groups associate in the aqueous medium to form a noncovalent bridge that functions much like a covalent bridge in stabilizing the duplex. Not unexpectedly, the systems differ in response to changes in concentration of the oligomers. Whereas the stability of a hairpin structure is independent of concentration,[3] association of the cholesteryl oligonucleotides decreases with decreasing oligomer concentration, as characteristic for bimolecular systems (T_m values for the complex in expt 6 are 52 and 46 °C at oligomer concentrations of 5.0 and 1.2 μM, respectively).

The importance of location of the hydrophobic groups was brought out by expts 12,13, in which the partners in expts 10,11 were exchanged. In these cases, hybridization was relatively poor, as shown both by reduced hyperchromicity and a lower dissociation temperature (Figure 2). On the other hand, a dT:dA oligomer pair containing cholesteryl groups in the "wrong" orientation exhibited a high T_m (expt 14). Since oligo(dA):oligo(dT) segments can form parallel-stranded duplexes,[7] a parallel alignment of the oligomers in which the cholesteryl groups overlap, as indicated in expt 14, Table I, is an attractive possibility. This structure is supported by the observation that the fluorescence

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(5) Krieg, A. M.; Tonkinson, J.; Matson, S.; Zhao, Q.; Saxon, M.; Zhang, L. Z.; Bhanja, U.; Yakubov, L.; Stein, C. A. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 1048–1052, and references therein.

(6) 5′-Terminal cholesterylphosphoryl groups (*ch′*) and internucleoside phosphoramidate links bearing a tethered cholesteryl fragment (*ch*) were introduced as described in refs 4b and 4a, respectively. For a related oxidative coupling see: Jäger, A.; Levy, M. J.; Hecht, S. M. *Biochemistry* **1988**, *27*, 7237–7246. Phosphodiester segments were prepared by standard solid-phase methodology manually or on a Milligen Cyclone synthesizer. Oligomers were characterized by RP-HPLC, PAGE, and melting curves of complexes formed with complementary oligomers by previously described procedures (ref 4 and Gryaznov, S. M.; Letsinger, R. L. *Nucleic Acids Res.* **1993**, *21*, 1403–1408).

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Table I. Effect of Cholesteryl Substituents on T_m Values of Oligonucleotide Complexes[a]

expt	oligomers	T_m (°C)	expt	oligomers	T_m (°C)
1	5′TTTTTTTTTT 3′AAAAAAAAA	16	10	5′C*ch*GATTGTTG 3′G*ch*CTAACAAC	69
2	5′TTTTTTTTT*ch*T 3′AAAAAAAAA	17	11	5′CGATTGTT*ch*G 3′GCTAACAA*ch*C	68
3	5′TTTTTTTTT*ch*T 3′AAAAAAAAA*ch′*	47	12	5′C*ch*GATTGTTG 3′GCTAACAA*ch*G	*b*
4	5′TTTTTTTTTT 3′AAAAAAAAAA	20	13	5′CGATTGTT*ch*G 3′G*ch*CTAACAAG	*b*
5	5′T*ch*TTTTTTTTT 3′AAAAAAAAA	20	14	5′A*ch*AAAAAAAAA 5′T*ch*TTTTTTTTT	52
6	5′TTTTTTTTT*ch*T 3′AAAAAAAAA*ch*A	52	15	5′TTTTTTTTT*ch*T 3′AAAAAAAAA*ch*A 3′TTTTTTTTT*ch*T	55
7	5′GATCCCGATTGTTG*ch*G 3′GCTAACAAC	35	16	5′T*ch*TTTTT] AAAAAA X 3′T*ch*TTTTT]	18
8	5′GATCCCGATTGTTG*ch*G 3′GCTAACAAC*ch′*	58	17	5′T*ch*TTTTT] 5′*ch*AAAAAA X 3′T*ch*TTTTT]	52
9	5′CGATTGTTG 3′GCTAACAAC	35			

[a] All compounds are deoxyribonucleotides. Abbreviations: *ch* designates the internucleoside link -OP(O)[NHCH$_2$CH$_2$NHC(O)O-cholesteryl]O-; *ch′*, the 5′ terminal substituent, cholesteryl-OP(O)(O$^-$)O-; -X-, the internucleotide bridge -OP(O)(O$^-$)O(CH$_2$)$_6$NHC(O)C$_6$H$_4$C(O)NH(CH$_2$)$_6$OP(O)-(O$^-$)O-. T_m is the temperature at the midpoint of the melting curve. The concentration are as follows: oligomer strands, 5 μM; NaCl, 0.10 M; Tris·HCl buffer, 10 mM (pH 7.0). [b] The transition was broad and weak, with T_m in the range of ~40–44 °C (see Figure 2).

of Hoechst-33258, a groove-binding drug, is enhanced to a greater extent by duplex **6** than by duplex **14** (3.5-fold), whereas the fluorescence of ethidium bromide, an intercalating drug, is enhanced to a greater extent by duplex **14** than by duplex **6** (1.3-fold).[8] These effects correspond to those reported for complexes containing anti-parallel and parallel oligo(dT):oligo(dA) segments in hairpin structures.[7]

Will hydrophobic substituents stabilize triple-stranded complexes? To probe this question, a titration of 3′AAA-AAAAAA*ch*A with 5′TTTTTTTTT*ch*T was carried out (see Figure 3A). A break in the absorbance plot occurred at the point where dT and dA oligomer concentrations were equal; no further break was found as the concentration of the thymidylate oligomer was increased. This result shows that the two compounds form a 1:1 complex but do not form a triple-stranded complex even in the presence of excess 5′TTTTTTTTT*ch*T. In contrast, when the titration was carried past the equivalence point by adding incremental amounts of 5′TTTTTTTTT*ch*T and then continued by adding incremental amounts of 3′TTTTTTTTT*ch*T, two addition changes in slope were found (Figure 3B). We conclude that 1 equiv of 3′TTTTTTTTT*ch*T binds to 1 equiv of the duplex generated in the first stage of the titration. A striking feature is the high stability of the mini triple strand; the complex obtained from stoichiometric amounts of the three oligomers in aqueous 0.10 M NaCl showed a single transition in an A_{260} *versus* temperature plot, with T_m 55 °C and hyperchromicity 48%. Significant hyperchromicity (37%) was likewise observed when the dissociation was followed at 280 nm, as characteristic for dT:dA triple-stranded polymers.[9] The unusual stability of the complex in expt 15 can be attributed to the fact that the hydrophobic substituents are in proximity when the oligomer strands are aligned appropriately to form triple strands.

Mini triplex structures can also be stabilized by an extended terephthalyl bridge.[3c] Some information on systems containing both terephthalyl and cholesteryl bridges was gained from expts 16,17. We found that cholesteryl groups did not further enhance binding of unmodified AAAAAA to the short, bridged thymidylate oligomer in expt 16 (T_m 18 °C, compared to T_m 20 °C for the same system without cholesteryl substituents[3c]); however, they led to a marked increase T_m when the target was 5′*ch′*AAAAAA (T_m 52 °C).

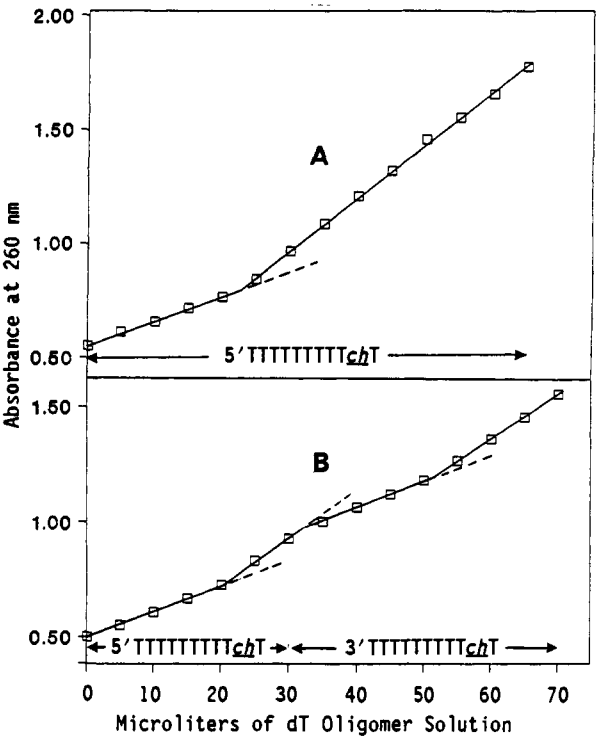

Figure 3. (A) Titration of 3′AAAAAAAAA*ch*A (5.3 nmol in 1.0 mL of solution) with 5′TTTTTTTTT*ch*T (0.24 mM, added in 5-μL aliquots); (B) titration of 3′AAAAAAAAA*ch*A (5.1 nmol in 1.0 mL of solution) using 5.0-μL aliquots of 0.24 mM 5′TTTTTTTTT*ch*T to a total of 30 μL, followed by 5.0-μL aliquots of 0.24 mM 3′TTTTTTTTT*ch*T to a total of 40 μL. All solutions were 0.10 M in NaCl and 10 mM in Tris·HCl buffer, pH 7.0, at room temperature.

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(8) Experimental conditions: aqueous 0.10 M NaCl, pH 7.10 (10 mM Tris·HCl), ~23 °C; each oligomer, 5 μM; Hoechst-33258 0.6 μM (λ_{ex} 355 nm, λ_{em} 480 nm); ethidium bromide 2 μM (λ_{ex} 525 nm, λ_{ex} 600 nm).

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