Formation of Chimeric Duplexes between Zwitterionic and Natural DNA

Hiromasa Hashimoto, Marek G. Nelson, and Christopher Switzer*
Department of Chemistry, University of California, Riverside, California 92521

Received April 29, 1993

Summary: We report a case where complete replacement of the natural nucleotides in a DNA strand with zwitterionic, ω-aminoalkylated ones does not inhibit duplex formation.

As part of our ongoing investigations on the properties of nonstandard oligonucleotides,[1,2] we report the synthesis of DNA that is rendered net charge neutral through replacement of thymidylate with zwitterionic nucleotides **1** or **2** bearing cationic base substituents (Figure 1). Oligonucleotides having diminished charge and enhanced resistance to nucleases are of current interest as antisense drugs that target mRNA.[3] In spite of the fact that hypermodified nucleotide bases are found naturally in tRNA and bacteriophage DNA,[4] base modification has been virtually ignored as a route by which to engineer oligonucleotides for use as antisense agents. Toward this end, it is known that bacteriophages use a variety of different hypermodified bases to protect their DNA from attack by phosphodiesterases present in their hosts.[5,6] Thus, bacteriophage ϕW-14 is known to replace approximately half of the thymines present in its DNA with positively charged α-putrescinylthymine to achieve such protection, resulting in one hypermodified base every eight nucleotides on average.[6] While it is known from these and other examples that the DNA major groove will tolerate some substitution without a significant deleterious effect on duplex formation, little information is available about the effect of introducing contiguous, bulky, charged modifying groups into the major groove.[7-9] We have addressed this issue and find that replacement of every nucleotide in a DNA dodecamer with a zwitterionic nucleotide results in a fully charge neutral oligomer with an unimpeded ability to form duplexes. This degree of major groove modification is unprecedented.

Table I. Properties of Zwitterionic Oligomers

oligodeoxynucleotide[a]	PAGE mobility[b]	50 mM NaCl[c] $\Delta G°_{37}$[d] (kcal/mol)	50 mM NaCl[c] T_m (°C)	T_m (°C) G-Mismatch[e]
5′-TTTTTTTTTTTT (4)	20.8	−4.2	22.5	11.5
5′-TTTTTTTTTTTT (5)	19.7	−4.4	22.5	
5′-TTTTTTTTTTTT (6)	15.5	−3.5	21.5	
5′-TTTTTTTTTTTT (7)	4.4	−3.6	19.0	13.5
		−3.8	18.5[f]	
5′-TTTTTTTTTTTT (8)	19.9	−4.0	22.0	
5′-TTTTTTTTTTTT (9)	16.3	−3.3	20.0	
5′-TTTTTtTtTtTt (10)	20.1	+0.50	15.5	

[a] "T", "T", and "t" are **1**, **2**, and **3**, respectively. [b] Polyacrylamide gel electrophoretic mobility is given in cm from the origin of the gel. The samples were electrophoresed in a 20% polyacrylamide, 7 M urea denaturing gel using Tris–borate–EDTA buffer at pH 8.5–9.0. [c] Sample contained 50 mM NaCl, 10 mM sodium phosphate, 0.1 mM EDTA, 2.5 μM $d(A)_{12}$, and 2.5 μM of **4**–**10** in H_2O at pH 7. [d] Derived from the average fitted parameters of melting curves from two different samples. [e] The following oligomer containing a G-mismatch was used in place of the complementary one: 5′-dAAAAGAAAAAAA. [f] Performed at pH 5.5.

We have prepared oligodeoxynucleotides **5**–**9** that are zwitterionic due to ω-(aminohexyl)- and ω-(aminopropyl)-uridylate zwitterions **1** and **2** (Figure 1 and Table I). As a control, oligodeoxynucleotide **10** containing 5-hexyl-2′-deoxyuridylate **3** which bears the hexyl tether used in zwitterion **1** without a terminal ammonium ion was also prepared (Figure 1 and Table I). Tethers three and six methylene groups in length were chosen because molecular models suggest a propyl tether is the minimum required to approximate the phosphate and ammonium ions, and natural precedent exists for an aza analog of a hexyl tether.[6] All oligonucleotides were synthesized from appropriately protected phosphoramidites using an automated DNA synthesizer.[10] Oligomers were characterized by digestion to constituent bases with formic acid followed by HPLC analysis,[11] 5′-end labeling followed by PAGE, and also by laser desorption mass spectrometry.

The gel electrophoretic mobilities of the pure oligomers, their melting temperatures in the presence of complementary (and mismatched) natural DNA, and their free energies of duplex formation are summarized in Table I.[12] The electrophoretic data shows an expected correspon-

(1) Hashimoto, H.; Switzer, C. *J. Am. Chem. Soc.* **1992**, *114*, 6255.
(2) Bain, J.; Switzer, C.; Chamberlin, A. R.; Benner, S. A. *Nature* **1992**, *356*, 537.
(3) For reviews, see: (a) Uhlman, E.; Peyman, A. *Chem. Rev.* **1990**, *90*, 543. (b) Riordan, M. L.; Martin, J. C. *Nature* **1991**, *350*, 442.
(4) Hall, R. H. *The Modified Nucleosides in Nucleic Acids*; Columbia University Press: New York, 1971.
(5) (a) Fleischman, R. A.; Campbell, J. L.; Richardson, C. C. *J. Biol. Chem.* **1976**, *251*, 1561. (b) Kaplan, D. A.; Nierlich, D. P. *J. Biol. Chem.* **1975**, *250*, 2395.
(6) (a) Kropinski, A. M. B.; Bose, R. J.; Warren, R. A. J. *Biochemistry* **1973**, *12*, 151. (b) Miller, P. B.; Wakarchuk, W. W.; Warren, R. A. J. *Nucleic Acids Res.* **1985**, *13*, 2559.
(7) Oligodeoxynucleotides bearing a base tethered ω-amino group have been used in the past to attach various probes to DNA, usually where one modified base is present per oligonucleotide, but never where all of the bases in an oligomer are modified. See, for example: (a) Povsic, T. J.; Strobel, S. A.; Dervan, P. B. *J. Am. Chem. Soc.* **1992**, *114*, 5934. (b) MacMillan, A. M.; Verdine, G. L. *Tetrahedron* **1991**, *47*, 2603. (c) Telser, J.; Cruickshank, K. A.; Morrison, L. E.; Netzel, T. L.; Chan, C. *J. Am. Chem. Soc.* **1989**, *111*, 7226. (d) Telser, J.; Cruickshank, K. A.; Schanze, K. S.; Netzel, T. L. *J. Am. Chem. Soc.* **1989**, *111*, 7221. (e) Allen, D. J.; Darke, P. L.; Benkovic, S. J. *Biochemistry* **1989**, *28*, 4601. (f) Gebeyehu, G.; Rao, P. Y.; SooChan, P.; Simms, D. A.; Klevan, L. *Nucleic Acids Res.* **1987**, *15*, 4513. (g) Haralambidis, J.; Chai, M.; Tregear, G. W. *Nucleic Acids Res.* **1987**, *15*, 4857. (h) Jablonski, E.; Moomaw, E. W.; Tullis, R. H.; Ruth, J. L. *Nucleic Acids Res.* **1986**, *14*, 6115. (i) Smith, L. M.; Fung, S.; Hunkapiller, M. W.; Hunkapiller, T. J.; Hood, L. E. *Nucleic Acids Res.* **1985**, *13*, 2399. (j) Bodnar, J. W.; Zempsky, W.; Warder, D.; Bergson, C.; Ward, D. C. *J. Biol. Chem.* **1983**, *258*, 15206.
(8) While bacteriophages are known to introduce bulky charged substituents into the major groove of duplex DNA (see refs 5 and 6) as a rule they replace or only partially replace one out of the four natural bases with a hypermodified one, resulting in at most one modified base every four nucleotides on average.
(9) Although the zwitterionic nucleotides in the present study might be considered a unimolecular counterpart to the bimolecular interaction of biogenic polyamines with DNA, it is not currently known with certainty whether polyamines bind predominantly to the major or minor groove of DNA, and therefore this may not prove to be the case. For some leading references, see: (a) Menger, F. M.; D'Angelo, L. L. *J. Org. Chem.* **1991**, *56*, 3467. (b) Schmid, N.; Behr, J.-P. *Biochemistry* **1991**, *30*, 4357. (c) Haworth, I. S.; Rodger, A.; Richards, W. G. *Proc. R. Soc. London B* **1991**, *244*, 107. (d) Mascotti, D. P.; Lohman, T. M. *Proc. Natl. Acad. Sci., U.S.A.* **1990**, *87*, 3142. (e) Feuerstein, B. G.; Pattabiraman, N.; Marton, L. J. *Nucleic Acids Res.* **1990**, *18*, 1271.
(10) A complete description of phosphoramidite synthesis and the preparation of oligonucleotides is given in the supplementary material.
(11) Adams, R. L. P.; McKay, E. L.; Craig, L. M.; Burdon, R. H. *Biochim. Biophys. Acta* **1979**, *563*, 72.

0022-3263/93/1958-4194$04.00/0

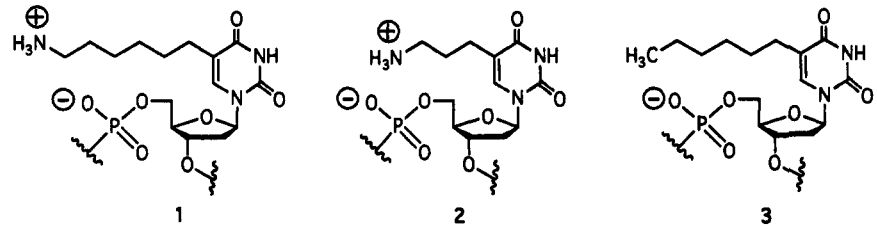

Figure 1.

dence between degree of zwitterionic nucleotide substitution and electrophoretic mobility, with the completely zwitterionic oligomer 7 traveling only a fraction of the distance of the other oligomers. The other data in Table I are discussed below.

In the presence of complementary DNA, zwitterionic oligomers 5–9 bearing one, four, or 11 aminohexyl zwitterions 1 or aminopropyl zwitterions 2 exhibit melting temperatures approximately equal to or slightly lower than the corresponding natural oligomer 4 (Table I). However, neither aminohexyl zwitterion 1 nor aminopropyl zwitterion 2 has an appreciable effect on the free energies of duplex formation for any of these oligomers (Table I). *In fact, even in the case of fully zwitterionic oligomer 7 with 11 contiguous aminohexyl zwitterions 1, duplex stability is not significantly affected.* To ensure that all of the amino groups present in the duplex of 7 are in their protonated form at pH 7 where the experiments were performed, an experiment was also conducted at pH 5.5 with essentially no change in the results (oligomer 7, second entry, Table I).

The influence on duplex stability by the positively charged ammonium ions of oligomers 5–9 may be assessed by comparing their stabilities with that of an oligomer with nucleotides bearing alkyl groups alone lacking ammonium ions. Thus, comparison of duplex stability for oligomer 10 bearing four hexyluridylates 3 ($\Delta G°_{37}$ = +0.5) with that of oligomer 6 bearing four aminohexyluridylates 1 ($\Delta G°_{37}$ = −3.5) shows clearly that the hexyl tethers exert a destabilizing effect in duplexes of oligomers 5–7 and the ammonium ions exert a stabilizing one. The difference in free energies between duplexes of oligomers 6 and 10, $\Delta\Delta G°_{37}$ = −4.0 kcal/mol, allows an estimation of the stabilizing influence of each ammonium ion in the duplex of 6 as −1 kcal/mol at 37 °C.

As expected for recognition via Watson–Crick base pairing, fully zwitterionic oligomer 7 gives a depressed T_m value mirroring that of natural oligomer 4 when zwitterion 1 is opposed by a G mismatch in the complementary DNA strand (Table I). The stoichiometry of complexes of

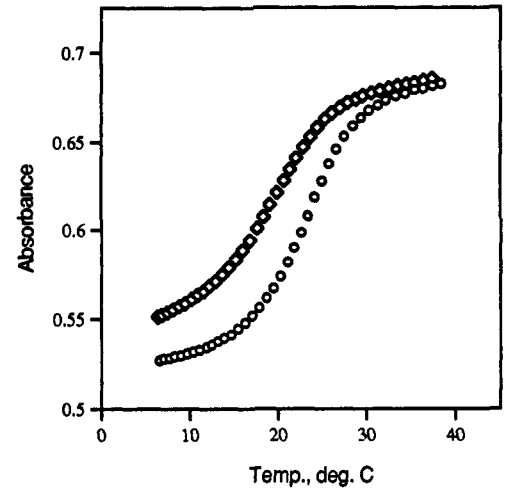

Figure 2. UV absorbance profiles versus temperature for 5′-d(T)$_{12}$ (○) and 5′-dTTTTTTTTTTTT (◇) in the presence of 5′-d(A)$_{12}$. T is 1. Experimental conditions were as noted in the legend of Table I, footnote c.

oligomers 4 and 7 with complementary DNA was determined in each case to be 1:1 from UV mixing curves.[13] UV absorbance versus temperature curves from which T_m and thermodynamic data were derived for these duplexes are presented in Figure 2.

We are currently evaluating the effects of different tether structures on the ability of zwitterionic oligonucleotides of the type reported here to complex both single- and double-stranded DNA and RNA.

Acknowledgment. This work was supported by the California Universitywide AIDS Research Program and the National Institutes of Health (GM-47375). We thank Gary Hathaway at the University of California, Riverside, Biotechnology Instrumentation Facility for obtaining mass spectra of oligonucleotides.

Supplementary Material Available: Procedures and compound characterization data (13 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(12) Melting temperatures and free energies of duplex formation were determined by nonlinear regression fitting of melting curves according to a two-state model using the method of Turner: Longfellow, C. E.; Kierzek, R.; Turner, D. H. *Biochemistry* **1990**, *29*, 278.

(13) Felsenfeld, G.; Rich, A. *Biochim. Biophys. Acta* **1957**, *26*, 457.