Triplex Formation by an Oligonucleotide Containing N^4-(3-Acetamidopropyl)cytosine

Chin-Yi Huang, Cynthia D. Cushman, and Paul S. Miller*

Department of Biochemistry, School of Hygiene and Public Health, The Johns Hopkins University, 615 North Wolfe Street, Baltimore, Maryland 21205

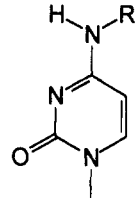
Received May 28, 1993®

Summary: Triplex formation has been observed between an oligodeoxyribonucleotide duplex and an oligodeoxyribopyrimidine which contains N^4-(3-acetamidopropyl)cytosine, a novel base analog which selectively interacts with a C·G base pair of the target duplex.

Oligodeoxyribopyrimidines can interact with the homopurine strand of an oligodeoxyribopurine·oligodeoxyribopyrimidine duplex through the formation of C^+·G·C and T·A·T base triads.[1-4] The presence of a single C·G or T·A base pair in the otherwise purine·pyrimidine sequence motif of the target duplex can be accommodated by naturally occurring bases in the third strand, although such "mismatches" generally lead to overall reduced stability of the triplex.[2-6] An exception to this is the observation that G can recognize a single T·A base pair.[1f,g,3,5,7,8] Recently, Griffin *et al.* described a nonnatural base, 4-(3-benzamidophenyl)imidazole, which interacts selectively with C·G and T·A base pairs.[9,10] 2′-Deoxynebularine, when incorporated into a third-strand oligopyrimidine, has been reported to bind to C·G base pairs and to also interact with A·T base pairs of a target duplex.[11] We have studied triplex formation in the oligodeoxyribonucleotide system shown in Figure 1. Oligomer **I(*X*)** contains thymine, 5-methylcytosine, C, and N^4-modified cytosine, X. The structures of the modified bases are shown in the lower part of Figure 1. Oligomers containing N^4-(butyl)C, **I(2)**, N^4-(3-carboxypropyl)C, **I(3)**, or N^4-(3-aminopropyl)C, **I(4)**, were prepared by reaction of oligomer **I(1)** with the appropriate amine in the presence of sodium bisulfite. This reaction selectively transaminates cytosine but does not modify 5-methylcytosines because 5-methylcytosine, unlike cytosine, does not form an adduct with bisulfite.[12] Oligomer **I(5)**, which contains an N^4-(3-acetamidopropyl)C, was prepared by reaction of **I(4)** with p-nitrophenyl acetate in acetonitrile solution in the presence of triethylamine. Control experiments showed that this treatment does not modify 5-methyldeoxycytidine nor does it acetylate the 5′- or 3′-hydroxyl groups of the nucleoside. The modified oligomers were completely hydrolyzed by treatment with a combination of snake venom phosphodiesterase and bacterial alkaline phosphatase and gave the expected nucleosides, including the appropriate C-modified nucleoside.[12]

Interactions between oligomer **I(*X*)** and duplex **II·III-(Y·Z)** were studied by recording the A_{260} *vs* temperature profile over the temperature range 0–60 °C. These melting experiments were carried out in a buffer containing 0.1 M sodium chloride, 20 mM magnesium chloride, and 50 mM

d-CTTCTTTTTTXTTTT I
d-GAAGAAAAAAYAAAA II
CTTCTTTTTTZTTTT-d III



1 R = H
2 R = -$(CH_2)_3CH_3$
3 R = -$(CH_2)_3COOH$
4 R = -$(CH_2)_3NH_2$
5 R = -$(CH_2)_3NHC(O)CH_3$

Figure 1. Sequences of the triplex-forming oligodeoxyribonucleotides **I**, **II**, and **III**. C is 5-methylcytosine, and the structures of the N^4-modified cytosines, X, are shown below the sequences.

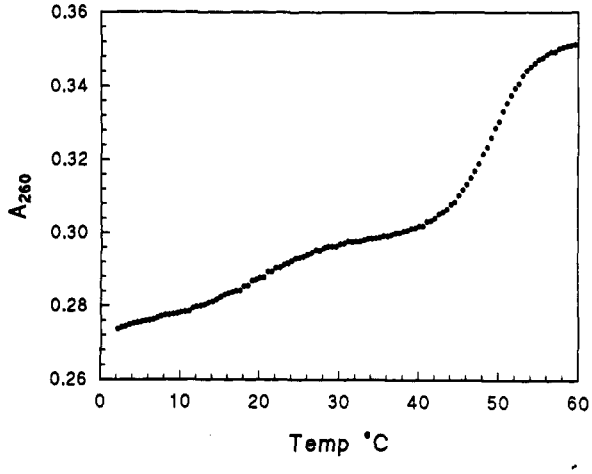


Figure 2. Melting profile of **I·II·III(5-C-G)**. The buffer contained 0.1 M sodium chloride, 20 mM magnesium chloride, 50 mM Tris, pH 7.0, and the strand concentration was 1 μM per strand. The solution was heated at 0.5 °C/min.

® Abstract published in *Advance ACS Abstracts*, September 1, 1993.

(1) See, for example: (a) LeDoan, T.; Perrouault, L.; Praseuth, D.; Habhoub, N.; Decout, J.-L.; Thuong, N. T.; Lhomme, J.; Helene, C. *Nucleic Acids Res.* **1987**, *15*, 7749–7760. (b) Moser, H. E.; Dervan, P. B. *Science* **1987**, *238*, 645–650. (c) Francois, J.-C.; Saison-Behmoaras, T.; Barbier, C.; Chassigonol, M.; Thuong, N. T.; Helene, C. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *16*, 11431–11440. (d) Rajagopal, P.; Feigon, J. *Nature* **1989**, *339*, 637–640. (e) Rajagopal, P.; Feigon, J. *Biochemistry* **1989**, *28*, 7859–7870. (f) de los Santos, C.; Rosen, M.; Patel, D. *Biochemistry* **1989**, *28*, 7282–7288. (g) Radhakrishnan, I.; Gao, X.; de los Santos, C.; Live, D.; Patel, D. J. *Biochemistry* **1991**, *30*, 9022–9030.
(2) Macaya, R. F.; Gilbert, D. E.; Malek, S.; Sinsheimer, J. S.; Feigon, J. *Science* **1991**, *254*, 270–274.
(3) Mergny, J.-L.; Sun, J.-S.; Rougee, M.; Montenay-Garestier, T.; Barcelo, F.; Chomilier, J.; Helene, C. *Biochemistry* **1991**, *30*, 9791–9798.
(4) Roberts, R. W.; Crothers, D. M. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 9397–9401.
(5) Yoon, K.; Hobbs, C. A.; Koch, J.; Sardaro, M.; Kutny, R.; Weis, A. L. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *30*, 3840–3844.
(6) Miller, P. S.; Cushman, C. D. *Biochemistry* **1993**, *32*, 2999–3004.
(7) Griffin, L.; Dervan, P. B. *Science* **245**, 967–971.
(8) Wang, E.; Malek,S.; Feigon, J. *Biochemistry* **1992**, *31*, 4838–4846.
(9) Kiessling, L. L.; Griffin, L. C.; Dervan, P. B. *Biochemistry* **1992**, *31*, 2829–2834.
(10) Griffin, L. C.; Kiessling, L. L.; Beal, P. A.; Gillespie, P.; Dervan, P. B. *J. Am. Chem. Soc.* **1992**, *114*, 7976–7982.
(11) Stilz, H. U.; Dervan, P. B. *Biochemistry* **1993**, *32*, 2177–2185.
(12) Miller, P. S.; Cushman, C. D. *Bioconjugate Chem.* **1992**, *3*, 74–79.

0022-3263/93/1958-5048$04.00/0

Table I. Melting Temperatures of the Third Strands of Triplex I·II·III

base substitutions[a]			T_m[b] (°C)
X	Y	Z	strand I
1	C	G	<0
2	C	G	<0
3	C	G	<0
4	C	G	10
5	C	G	20
			19[c]
5	T	A	<0[c]
5	A	T	<0[c]
5	G	C	8[c]
1	G	C	32

[a] See Figure 1 for structures of modified bases. [b] Melting experiments were carried out in buffer containing 0.1 M sodium chloride, 20 mM magnesium chloride, 50 mM Tris·HCl, pH 7.0, at a concentration of 1 μM of each oligomer strand. [c] Melting experiments were carried out in buffer containing 0.1 M sodium chloride, 20 mM magnesium chloride, 50 mM 3-(*N*-morpholino)propanesulfonic acid (MOPS), pH 7.0, at a concentration of 1 μM of each oligomer strand.

Tris or 50 mM MOPS buffered at pH 7.0. The solutions were heated at a rate of 0.5°C/min. The melting profile for the complex **I·II·III(5·C·G)** in which oligomer **I** contains N^4-(3-acetamidopropyl)C, **I(5)**, is shown in Figure 2. Triplex formation is indicated by the presence of two transitions in the melting profile. The first, lower temperature, transition corresponds to melting of the third strand, **I(5)**, and the second, higher temperature, transition corresponds to melting of the duplex, **II·III(C·G)**.

As shown in Table I, triplex formation between **I(*X*)** and duplex **II·III(C·G)** was also observed when **I** contained N^4-(3-aminopropyl)C but not when **I** contained C, N^4-(butyl)C, or N^4-(3-carboxypropyl)C. These results suggest the side chains of N^4-(3-aminopropyl)C or N^4-(3-acetamidopropyl)C interact in a specific manner with the C·G base pair of the duplex. Examination of molecular models suggests that the length of the side chain is sufficient to allow either the 3-amino group hydrogens of N^4-(3-aminopropyl)C or the amide hydrogen of N^4-(3-acetamidopropyl)C to hydrogen bond to the O-6 carbonyl of G of the target C·G base pair. Consistent with this possibility is the failure to observe triplex formation by **I(2)** and **I(3)**, oligomers whose N^4-(butyl)C or N^4-(3-carboxypropyl)C side chains lack hydrogen bond donating groups.

Triplex formation between **I(5)** and **II·III(Y·Z)** appears to be selective for the C·G base pair as is shown by the data in Table I. Triplex formation was not observed when **Y·Z** was A·T or T·A. A triplex of low stability was observed when **Y·Z** was G·C. The third strand Tm of this triplex is 24 °C less than that observed for the triplex formed by **I·II·III(1·G·C)**. The apparent base pair selectivity by N^4-(3-acetamidopropyl)C observed in this system is in contrast to that reported for 4-(3-benzamidophenyl)imidazole and for 2′-deoxynebularine. In the case of 4-(3-benzamidophenyl)imidazole, shape-selective recognition was believed to be responsible for interaction of the base with C·G and T·A base pairs rather than specific hydrogen-bonding interactions.[10] In the case of nebularine, a single hydrogen bond was postulated to be involved in recognition of C·G and A·T base pairs.[11]

It is somewhat surprising that a base with a flexible side chain such as N^4-(3-acetamidopropyl)C can support triplex formation. The results suggest that modified bases with more rigid side chains might also be designed which could participate in triplex formation. Studies to explore this possibility and to further characterize the hydrogen bonding interactions of the N^4-(3-aminopropyl)C and N^4-(3-acetamidopropyl)C analogs are currently in progress.

Acknowledgment. These studies were supported by a grant from the National Institutes of Health, GM 45012. C.-Y.H. is currently a GENTA Fellow in the Department of Biochemistry, JHU.

Supplementary Material Available: Experimental procedures for the preparation and characterization of the oligonucleotides and for the melting experiments (3 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.