Effect of Third Strand Orientation on Oligonucleotide Intramolecular Triplex Stability

David M. Noll,† Anne-Frances Miller,‡ and Paul S. Miller*,†

Department of Biochemistry
School of Hygiene and Public Health
Department of Chemistry, Johns Hopkins University
Baltimore, Maryland 21205

Received June 4, 1996

Intramolecular nucleic acid triplexes provide useful model systems for studying the stability and structure of triple-stranded DNA[1] and the interactions of various ligands with triple-stranded DNA.[1g,2] In addition, intramolecular triplexes may have important biological functions in cellular DNA.[3] We have found that the stability of intramolecular DNA triplexes which have a pyrimidine·purine·pyrimidine binding motif depends strongly on whether the Hoogsteen binding domain precedes or follows the Watson−Crick domains in the molecule. This could have important implications for structural studies involving this type of molecule.

Two oligodeoxyribonucleotides shown in Figure 1, **I(C·G·C)** and **II(C·G·C)**, where C is 5-methyldeoxycytidine, were prepared.[4] Both oligomers contain the same six base triads. In oligomer **I(C·G·C)**, the Hoogsteen binding domain is located at the 5′-end of the oligomer and the purine binding domain is located at the 3′-end. Oligomer **II(C·G·C)** contains the same order of nucleotides as **I(C·G·C)**, but the polarity of the strand is reversed, which places the Hoogsteen binding domain at the 3′-end and the purine binding domain at the 5′-end.

The 1D proton NMR spectra (Figure 2) of both oligomers are consistent with the formation of triplexes. Thus, resonances with chemical shifts above 14.9 ppm and between 8.5 and 10.35 ppm are diagnostic of imino and amino protons, respectively, of protonated 5-methylcytosine residues involved in Hoogsteen hydrogen bonds.[1k] Eleven of the 12 expected Watson−Crick and Hoogsteen imino protons are seen between 12.5 and 16 ppm. That the 5-methylcytosine imino proton of the terminal **C·G·C** triad is not observed is most likely due to its rapid exchange with solvent.[1f]

The stabilities of these two intramolecular triplexes are quite different, as is apparent from the melting profiles shown in Figure 3A. Oligomer **I(C·G·C)** exhibits two distinct melting transitions: the first, whose T_m is 23 °C, represents dissociation

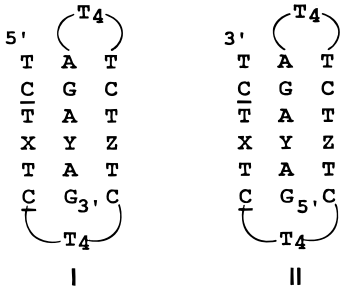

Figure 1. Intramolecular triplexes **I(X·Y·Z)** and **II(X·Y·Z)**. In this notation, X specifies the nucleotide in the Hoogsteen domain and Y and Z are nucleotides in the purine-rich and pyrimidine-rich domains, respectively.

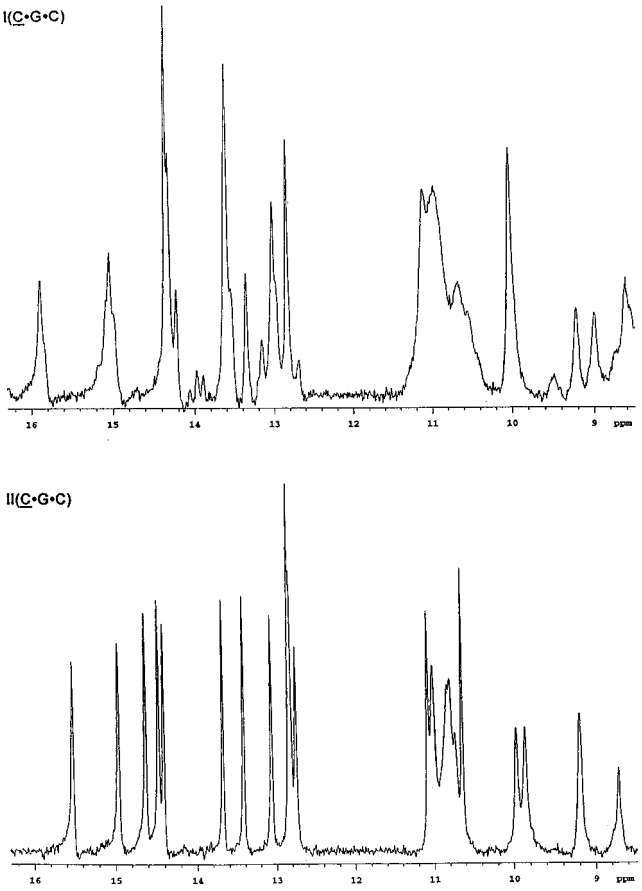

Figure 2. Imino and C^+ amino region of the 500 MHz proton NMR spectra of **I(C·G·C)** and **II(C·G·C)**. The NMR samples contained 0.75 mM **I(C·G·C)** or 0.55 mM **II(C·G·C)** in 10 mM sodium phosphate (pH 6.5),[5] 100 mM sodium chloride, and 90% H_2O/10% D_2O. Spectra were collected at 5 °C on a Varian Unity^{plus} 500 spectrometer using a symmetrically shifted pulse[6] to suppress solvent, with an excitation maximum of 12.4 ppm, a 14 000 Hz sweep width, a 0.5 s acquisition time, and 128 scans per spectrum. A 24° shifted sine bell window function was applied prior to Fourier transformation and base line correction.

† Address: Department of Biochemistry, School of Hygiene and Public Health, Johns Hopkins University, 615 North Wolfe St., Baltimore, MD 21205.

‡ Department of Chemistry.

(1) (a) Radhakrishnan, I.; de los Santos, C.; Live, D.; Patel, D. J. *Biochemistry* **1991**, *30*, 9022−9030. (b) Wang, E.; Malek, S.; Feigon, J. *Biochemistry* **1992**, *31*, 4838−4846. (c) Macaya, R.; Wang, E.; Schultze, P.; Sklenar, V.; Feigon, J. *J. Mol. Biol.* **1992**, *225*, 755−773. (d) Radhakrishnan, I.; de los Santos, C.; Patel, D. J. *J. Mol. Biol.* **1993**, *234*, 188−197. (e) Koshlap, K. M.; Gillespie, P.; Dervan, P. B.; Feigon, J. *J. Am. Chem. Soc.* **1993**, *115*, 7908−7909. (f) Radhakrishnan, I.; Patel, D. J. *J. Mol. Biol.* **1994**, *241*, 600−619. (g) Radharkrishnan, I.; Patel, D. J. *Biochemistry* **1994**, *33*, 11405−11416. (h) Bornte, O.; Lancelot, G.; Chanteloup, L.; Thuong, N. T.; Beau, J. M. *J. Biomol. NMR* **1994**, *4*, 575−580. (i) Volker, J.; Klump, H. H. *Biochemistry* **1994**, *33*, 13502−13508. (j) Jiang, S. P.; Jernigan, R. L.; Ting, K. L; Syi, J. L.; Raghunathan, G. *J. Biomol. Struct. Dyn.* **1994**, *12*, 383−399. (k) Feigon, J.; Koshlap, K. M.; Smith, F. W. *Methods Enzymol.* **1995**, *261*, 225−255. (l) Klinck, R.; Liquier, J.; Taillandier, E.; Goyette, C.; Huynh-Dinh, T.; Guittet, E. *Eur. J. Biochem.* **1995**, *233*, 544−553. (m) Bornet, O.; Lancelot, G. *J Biomol. Struct. Dyn.* **1995**, *12*, 803−814. (n) Wang, Y.; Patel, D. J. *Biochemistry* **1995**, *34*, 5696−5704. (o) Plum, G. E.; Breslauer, K. J. *J. Mol. Biol.* **1995**, *248*, 679−695. (p) Dagneaux, C.; Liquier, J.; Taillandier, E. *Biochemistry* **1995**, *34*, 14815−14818.

(2) (a) Durand, M.; Thuong, N. T.; Maurizot, J. C. *Biochimie* **1994**, *76*, 181−186. (b) Durand, M.; Thuong, N. T.; Maurizot, J. C. *J. Biomol. Struct. Dyn.* **1994**, *11*, 1191−1202.

(3) Bacolla, A.; Ulrich, M. J.; Larson, J. E.; Ley, T. J.; Wells, R. D. *J. Biol. Chem.* **1995**, *270*, 24556−24563.

(4) The oligonucleotides were synthesized by standard procedures on controlled pore glass supports using protected nucleoside 3′-*O*-*β*-cyanoethyl-*N*,*N*-diisopropylphosphoramidites. The oligomers were deprotected by treatment with concentrated ammonium hydroxide/pyridine solution (1:1 v/v) at 55 °C for 12 h and were purified by reversed phase HPLC. The extinction coefficients of the oligomers were determined following digestion with snake venom phosphodiesterase as previously described. Miller, P. S.; Cushman, C. D. *Biochemistry* **1993**, *32*, 2999−3004.

(5) This is the lowest pH at which two distinct transitions can be seen in the UV melting profiles of **I(C·G·C)** and **II(C·G·C)**.

(6) Smallcombe, S. H. *J. Am. Chem. Soc.* **1993**, *115*, 4776−4785.

S0002-7863(96)01881-1 CCC: $12.00

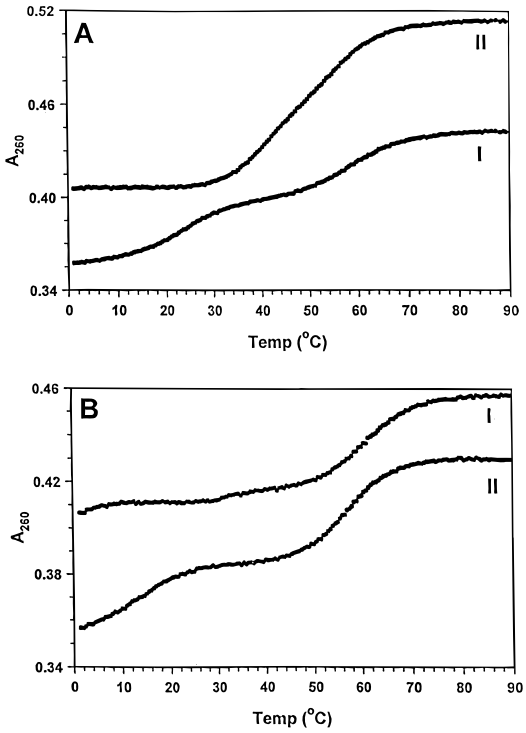

Figure 3. Absorbance *vs* temperature profiles of (A) **I(C·G·C)** or **II(C·G·C)** and (B) **I(U·C·G)** or **II(U·C·G)**. Solutions containing 2 μM oligomer in 10 mM sodium phosphate (pH 6.5)[5] and 100 mM sodium chloride were heated at a rate of 0.5 °C/min.

of the Hoogsteen domain and the second, whose T_m is 59 °C, represents dissociation of the Watson−Crick duplex.[1i] Close inspection of the melting profile of oligomer **II(C·G·C)** reveals the presence of two transitions whose total hypochromicity is slightly greater than that of **I(C·G·C)**.[7] The T_m for dissociation of the Hoogsteen domain of this oligomer is 41 °C, which is 18 °C higher than that of the corresponding transition in **I(C·G·C)**.

Further examination of the NMR spectra recorded at 5 °C, which is well below the T_m for third strand dissociation (see Figure 2), suggests that **I(C·G·C)** is less ordered than **II(C·G·C)**. Specifically the spectrum of **II(C·G·C)** displays well-resolved resonances of uniformly narrow line widths, whereas that of **I(C·G·C)** has generally broader line widths and multiple, smaller resonances indicative of minor conformers. This, in conjunction with the decrease in the total hypochromicity, is consistent with **I(C·G·C)** adopting a less-ordered, more dynamic structure.

Strand orientation plays a particularly important role in the stability of intramolecular triplexes which contain a **U·C·G** triad such as **I(U·C·G)** and **II(U·C·G)**. Thus, as shown in Figure 3B, oligomer **I(U·C·G)** does not form a triplex above 0 °C, whereas the T_m for dissociation of the Hoogsteen domain of **II(U·C·G)** is 13 °C.

Previous studies on bimolecular and circular triplex-forming oligodeoxyribonucleotides have shown that the size and composition of the loops in these molecules can affect overall triplex stability.[8] In the present intramolecular system, the composition of the loop connecting the two pyrimidine domains is the same in both oligomers. However evidence for the role of T_4 loops in stabilizing **II(C·G·C)** can be found by examining the line shapes of the imino resonances of the loop thymidines between 10.5 and 11.5 ppm. In **II(C·G·C)** the line widths of many of the T_4 imino protons are as sharp or sharper than the imino and amino base-paired resonances. This suggests that the loops are as well-ordered as the rest of the structure and as such can contribute to the overall stability of the triplex. In contrast, the line widths of the T_4 imino proton resonances of **I(C·G·C)** are significantly broader, indicative of a more dynamic and less stable structure. These differences in loop structure may be due to different stacking interactions of the T_4 loops because in oligomer **I** the thymidines of the loop can stack on the 3′-side of the Hoogsteen binding domain, whereas in **II** they can stack on the 5′-side. This difference in loop stacking orientation may account for the remarkable differences in stabilities of the two types of triplexes.

The above results demonstrate that strand orientation is an important parameter to consider when designing intramolecular triplexes for physical studies, especially those containing novel base triads.

Acknowledgment. The authors thank Ms. Sarah Kipp for assistance in preparing the oligonucleotides. This work was supported by a grant from the National Institutes of Health (GM 45012). D.M.N. is supported by a training grant from the National Institute of Environmental Health Sciences (5T32 ES07141).

JA9618816

(7) First derivative analysis of the data shows two maxima with T_m values of 40.9 and 52.5 °C.

(8) (a) Parkash, G.; Kool, E. T. *J. Am. Chem. Soc.* **1992**, *114*, 3523−3527. (b) Wang, S.; Booher, M. A.; Kool, E. T. *Biochemistry* **1994**, *33*, 4639−4644.