Studies on the Extension of Sequence-independence and the Enhancement of DNA Triplex Formation

Ming-Tsai Wei[a] (魏明財), Axel Walter[b], Anna Gabrielian[c], Holger Schütz[b],
Eckhard Birch-Hirschfeld[d], Shwu-Bin Lin[e] (林淑萍), Wei-Chen Lin[a#] (林維政),
Hartmut Fritzsche[b] and Lou-Sing Kan[a]* (甘魯生)
[a]*Institute of Chemistry, Academia Sinica, Taipei, Taiwan, R.O.C.*
[b]*Institute of Molecular Biology, Friedrich Schiller University, Jena, Germany*
[c]*Institute of Molecular Biology, Armenian Academy of Sciences, Yerevan, Armenia*
[d]*Institute of Virology, Friedrich Schiller University, Jena, Germany*
[e]*The Graduate Institute of Medical Technology, National Taiwan University, Taipei, Taiwan, R.O.C.*

A more elaborate sequence-independent triple-helix formation viability study was carried out and extended from a recombination-like triple-helical DNA motif of a previous study (J. Mol. Recognition 14, 122-139 (2001)). The intended triple-helix was formed by mixing one part of a DNA hairpin duplex and one part of a single (or third) strand identical to one of the duplex strands and complementary to the other strand. In contrast to the common purine and pyrimidine motifs in triple-stranded DNA, the strands of the recombination-like motif are not monotonously built from pyrimidine only, or purine only, in the sequence. The stability of the recombination-like motif triplexes with varying sequences was monitored by UV thermal melting curves. The results showed that the order of the stability of the R-form DNA base triads (J. Mol. Biol., 239, 181-200 (1994)) is G*(GB C) > C*(CB G) > A*(AB T) > T*(T B A) (the Watson-Crick base pair is denoted in the parentheses) in 200 mM NaCl, at pH 7. In an attempt to increase the stability of the triplex in the recombination-like motif, we replaced cytidine by 5-methylcytidine (^{m}C) of the third strand. There is a general trend that ^{m}C modification stabilizes the complex (< 2 °C per ^{m}C). The complex is furthermore stabilized by Mg^{2+} ion. The Tm increases from 7 to 2 °C from less stable to highly stable triplex by 20 mM Mg^{2+} ion in solution.

Keywords: DNA triplex; Recombination-like motif; Parallel homologous strands; Base modification; Thermal melting.

INTRODUCTION

DNA can form triple-stranded helices[1-3] as reviewed in several articles.[4-8] There is a growing interest in triplex DNA because of its potential use in diagnostics or therapeutics in the so-called antigene strategy.[8-10] The antigene strategy has been shown to work *in vivo*.[11] However, the potential of the above biological applications is severely hindered by the strict dependence on the sequence. In either pyrimidine-purine- pyrimidine or purine-purine-pyrimidine type triplex, a purine clustered motif in the sequence is needed. Hence there is an on-going effort searching for conditions to relieve the stringent condition of triplex formation. A theoretical conformational analysis predicted that DNA triplexes might be formed without sequence restrictions[12] in which the third strand is identical to one strand of the Watson-Crick duplex and is located in the major groove of the duplex. Therefore, it is imperative to know the relative stabilities of the four possible R-form DNA base triads, A*(AB T), G*(G B C), T*(T B A) and C*(C B G) (the Watson-Crick duplex base pair is in the parentheses). In a previous paper, we described a 1:1 recombination-like triplex of a DNA duplex and a third strand, where the third strand has a parallel orientation and has an identical sequence to one of the strands of the hairpin.[13] Two of the four possible triads, A*(A B T) and G*(G B C), have been established in that report. A similar, but intra- molecular, parallel motif A*(A B T) was described by Dagneaux et al.[14] Recently, Shchyolkina et al. reported a protein-free par-

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* Corresponding author. Fax: +886-2-2788-4184; E-mail: lskan@chem.sinica.edu.tw

[#] Present address: The Genome Center, Academia Sinica, Taipei, Taiwan, R.O.C.

Abbreviations used: UV: ultraviolet spectroscopy, ^{m}C: 5-methylcytidine.

allel triple-stranded DNA.[15] In the current paper, we extended the previous study to a new recombination-like triplex composed of two 12-nucleotide (12-nt) complementary strands with Watson-Crick base pairing linked by four cytidines forming hairpin duplexes and 12-nt single strands of identical sequence to the purine-rich strand of the hairpin duplex (Scheme I and Table 1). The cytidine of the 12-nt third-stranded oligomer is subject to modification by ^{m}C (Table 1). The triplexes were prepared by 1:1 mixing of the duplex and a third-strand (Scheme I and Table 1).

There fore, the sequence of triplex contains both purine and pyrimidine nucleotides. We studied the stabilities of all

Scheme I Triplexes are constructed by a hairpin duplex (with a C$_4$ loop) and a third-stranded oligodeoxyribonucleotide. The sequence of the latter one (from 5′ to 3′) is identical to one of the motifs of the hairpin duplex (in bold letters)

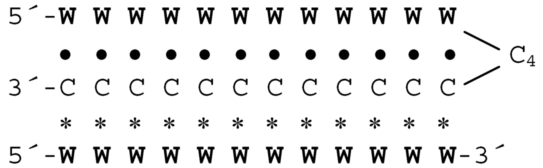

Table 1. The sequences of synthetic oligodeoxyribonucleotide triplexes are satisfied the criteria in Scheme I

Symbols	Sequences	Sequences in the same format of Scheme I
C3T3A6	$5'\text{-}\mathbf{C_3T_3A_6} + 5'\text{-}\mathbf{C_3T_3A_6}C_4T_6A_3G_3$	5′-**C C C T T T A A A A A A** C C 3′-G G G A A A T T T T T T C C 5′-**C C C T T T A A A A A A**
(CT)3A6	$5'\text{-}\mathbf{(CT)_3A_6} + 5'\text{-}\mathbf{(CT)_3A_6}C_4T_6(AG)_3$	5′-**C T C T C T A A A A A A** C C 3′-G A G A G A T T T T T T C C 5′-**C T C T C T A A A A A A**
C3A9	$5'\text{-}\mathbf{C_3A_9} + 5'\text{-}\mathbf{C_3A_9}C_4T_9G_3$	5′-**C C C A A A A A A A A A** C C 3′-G G G T T T T T T T T T C C 5′-**C C C A A A A A A A A A**
^{m}C3A9	$5'\text{-}\mathbf{{}^{m}C_3A_9} + 5'\text{-}\mathbf{C_3A_9}C_4T_9G_3$	5′-**C C C A A A A A A A A A** C C 3′-G G G T T T T T T T T T C C 5′-**^{m}C ^{m}C^{m}C A A A A A A A A A**
C5A7	$5'\text{-}\mathbf{C_5A_7} + 5'\text{-}\mathbf{C_5A_7}C_4T_7G_5$	5′-**C C C C C A A A A A A A** C C 3′-G G G G G T T T T T T T C C 5′-**C C C C C A A A A A A A**
(CA)5A2	$5'\text{-}(CA)_5A_2 + 5'\text{-}(CA)_5A_2C_4T_3(GT)_5$	5′-**C A C A C A C A C A A A** C C 3′-G T G T G T G T G T T T C C 5′-**C A C A C A C A C A A A**
g(^{m}CA)5A2	$5'\text{-}\mathbf{({}^{m}CA)_5A_2} + 5'\text{-}\mathbf{(CA)_5A_2}C_4T_3(GT)_5$	5′-**C A C A C A C A C A A A** C C 3′-G T G T G T G T G T T T C C 5′-**^{m}C A C A^{m}C A C A^{m}C A A A**
C3G3A6	$5'\text{-}\mathbf{C_3G_3A_6} + 5'\text{-}\mathbf{C_3G_3A_6}C_4T_6C_3G_3$	5′-**C C C G G G A A A A A A** C C 3′-G G G C C C T T T T T T C C 5′-**C C C G G G A A A A A A**
(CG)3A6	$5'\text{-}\mathbf{(CG)_3A_6} + 5'\text{-}\mathbf{(CG)_3A_6}C_4T_6(CG)_3$	5′-**C G C G C G A A A A A A** C C 3′-G C G C G C T T T T T T C C 5′-**C G C G C G A A A A A A**

four R-form base triads in triplexes formed between DNA duplex hairpins and the corresponding third strands with parallel orientation in alternate sequences (Scheme I and Table 1). Our overall design strategy is shown in Table 1. First, three each of T*(T B A) and C*(C B G) are introduced in triplexes C3T3A6 and (CT)3A6, both containing six A*(A B T). They have the same component triads but in different sequential orders. Namely, the former one has a (C)$_3$, and a (T)$_3$ motif, but the latter one has an alternating (CT)$_3$ sequence. Next, in the triplex C3A9, A*(A B T) has replaced the three T*(T B A)'s base triads of those in C3T3A6. Thus, the relative stabilities of T*(T B A) and A*(A B T) can be studied. Whereas in C5A7 and (CA)5A2, the number of C*(C B G) is increased by two, but the number of A*(A B T) is decreased by two, as compared to C3A9. Therefore, the relative stabilities of C*(CB G) and A*(A B T) can be revealed. Finally, we have introduced three G*(G B C)'s to replace three A*(A B T)'s in C3G3A6 and (CG)3A6. The property of G*(G B C) can be studied this way. The composition of different base triads in recombination-like triplexes listed in Table 1 is shown in Table 2 for clarity. Therefore, the stabilities of all four base triads could be studied systematically. In addition, we examined the enhancement of triplex stability of replacing cytidine by 5-methylcytidine (^{m}C) in the third-stranded (^{m}C3A9 and (^{m}CA)5A2 only) oligomer (Table 3). Similar to the effect in the well-known parallel motif pyrimidine-purine-pyrimidine,[16,17] substitution of cytidine by ^{m}C in the third-strand stabilizes the recombination-like triplex. The presence of the Mg^{2+} in solution serves the same purpose.

Table 2. The number of four base triads in recombination-like triplexes without ^{m}C

	T*(T B A)	A*(A B T)	C*(C B G)	G*(G B C)
C3T3A6	3	6	3	0
(CT)3A6	3	6	3	0
C3A9	0	6+3	3	0
C5A7	0	6+1	3+2	0
(CA)5A2	0	6+1	3+2	0
C3G3A6	0	6	3	3
(CG)3A6	0	6	3	3

Table 3. The melting temperatures T_m's of recombination-like triplexes and the hairpin duplex. The concentrations of NaCl and Mg^{2+} are described in the "Materials and Methods"

	Triplexes				Hairpin
	T_{m1} (°C)		T_{m2} (°C)		T_m (°C)
	NaCl	Mg^{2+}	NaCl	Mg^{2+}	NaCl
C3T3A6	--	--	63.3	65.3	64.3
(CT)3A6	36.1	43.1	63.5	66.1	64.3
C3A9	40.5	45.8	66.6	68.8	67.6
^{m}C3A9	43.3	48.7	67.1	69.7	68.0
C5A7	44.9	49.6	71.4	72.2	72.5
(CA)5A2	48.5	50.4	74.8	74.0	76.4
(^{m}CA)5A2	53.2	54.2	75.0	73.6	75.2
C3G3A6	51.1	50.5	75.4	75.0	75.8
(CG)3A6	56.3	58.2	79.8	78.6	81.0

MATERIALS AND METHODS

Materials

The ^{m}C phosphoramidite was purchased from Glen Research (Sterling, VA). All unmodified and ^{m}C contained oligodeoxynucleotides listed in Table 1 were synthesized by using automated phosphoramidite chemistry on a DNA synthesizer, purified, if necessary, by HPLC and desalted with a RP column as described previously.[13]

Optical Spectroscopy

UV spectroscopic experiments were performed with both Cary 100 and Cary 3E spectrometers (Varian Associates, Palo Alto, CA), and a M400 UV-VIS spectrophotometer (Carl Zeiss, Jena, Germany) equipped with a temperature controller and a thermostated cell holder. Melting profiles (absorbance vs. temperature) were collected at 260 nm from 11 to 91 °C at 0.2 °C increments with a heating rate of 1.0 °C/min. Solution conditions were 200 mM of NaCl, 20 mM of MgCl$_2$, 10 mM of sodium cacodylate buffer, and 0.1 mM of EDTA, pH 7.3, if not otherwise mentioned. The strand concentration was determined from the absorbance measured at 260 nm and at a temperature of 80 °C according to Sambrook et al.[18] All reported melting temperatures (T_m), determined from the first derivatives of melting profiles, are related to 2.0 μM strand concentration.

RESULTS

The biphasic UV melting curves reveal the relative stability of four base triads

All 1:1 recombination-like triplexes between DNA duplexes and the corresponding third-strands in Table 1 have been studied by UV thermal melting temperature. In most cases (except for C3T3A6), we observed a biphasic UV melting curve. A typical example (C3A9) of melting temperature measurement is shown in Fig. 1. The transitions at higher temperatures (T_{m2}) of the triplexes, including T3C3A6, were equal to their corresponding constituted hairpin duplexes within error range (1 °C on average). Therefore, the T_{m2} can be recognized as the dissociation/association of the hairpin portion in the recombination-like triplexes. It is plausible that the lower melting temperature (T_{m1}) can be assigned to the dissociation/association of the 1:1 recombination-like triplex into its components (Table 3).

Except for C3T3A6, the T_{m1} of the triplexes can be analysed by the compositions of their individual base triads. Both C3T3A6 and (CT)3A6 contain six A*(A B T), three T*(T B A), and three C*(C B G) base triads. However, the former did not form triplex, whereas the latter showed the lowest T_{m1} in Table 3. While the three T*(T B A)'s in C3T3A6 switched to three A*(A B T)'s, the melting temperature of C3A9 is higher than that of (CT)3A6. Since the C's and A's are in clusters, but not alternating, one can conclude that the formation of A*(A B T) is stronger than T*(T B A) thermodynamically. When two A*(A B T)'s in C3A9 are further replaced by C*(C B G)'s to form C5A7, it has an even higher T_m. One can again conclude that the stability of C*(C B G) is higher than that of A*(A B T). Finally, two C*(C B G)'s and one A*(A B T) in C5A7 have been replaced by three G*(G B C)'s in C3G3A6. Its T_{m1} is higher than that of C5A7. Therefore, the triad formation ability of G*(G B C) is better than C*(C B G). To summarize, the relative thermodynamic formation stability of the above four base triads can be expressed as:

G*(C B C) > C*(C B G) > A*(A B T) > T*(T B A).

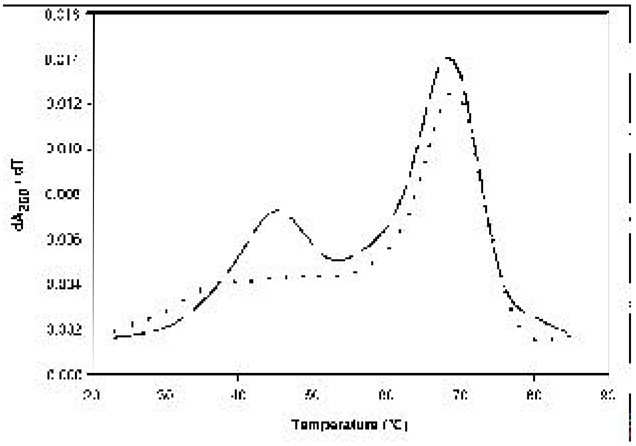

Fig. 1. UV melting curves of the C3A9 and of the hairpin duplex component. Only the first derivative of the UV melting curves are shown. C3A9: dashed line; the hairpin duplex ($5'$-C$_3$A$_9$C$_4$T$_9$G$_3$): dotted line. The experimental conditions are described in the 'Materials and Methods'.

The effect of sequence order on the formation of recombination-like triplex

Among C3T3A6/(CT)3A6, C5A7/(CA)5A2, and C3G3A6/(CG)3A6 triplex pairs have exactly the same base triad compositions but showed different T_{m1}'s (Table 3). This difference should have originated from the order of the sequence and the associated nearest neighbor stacking effect.[19] According to the analysis of Allawi and Santa Lucia, Jr.,[20] the ΔG of (all in 5′ to 3′) CCCTTTAAAAAA/CTCTCTAAAAAA, CCCCCAAAAAAA/CACACACACAAA, and CCCGGGAAAAAA/CGCGCGAAAAAA are, in duplex formation, -12.02/-12.54, -14.81/-15.01, and -15.83/-17.29 Kcal/mol. The formation free energy of CTCTCTAAAAAA, CACACACACAAA, and CGCGCGAAAAAA, with alternating sequences, are more favorable than those of CCCTTTAAAAAA, CCCCCAAAAAAA, and CCCGGGAAAAAA, respectively. To eliminate the common segments of A's, the duplex formation free energy of alternating sequence is more favored than those in clustered sequence. According to our results, the stability of the recombination-like triplex formation is also governed by the sequence.

Stabilization of the complex by ^{m}C

In C3A9 and (CA)5A2, the three C's of the third strand of the oligomer are replaced by ^{m}C to become 5′-^{m}C ^{m}C ^{m}CAAAAAAAAA (^{m}C3A9) and 5′-^{m}CACA ^{m}CACA ^{m}CAAA ((^{m}CA)5A2), respectively. The three ^{m}C's of the former are clustered, but not in the latter. Compared to the unmodified systems C3A9 and (CA)5A2, the T_{m1} of the UV melting curve of ^{m}C3A9 and (^{m}CA)5A2 increased by 2.8 (without Mg^{2+})/2.9 (with 20 mM Mg^{2+}) and 4.7 (without Mg^{2+})/3.8 °C (with 20 mM Mg^{2+}), respectively (Table 3). These findings correspond to the behavior of the C*(C B G) triads in the known pyrimidine-purine-pyrimidine motif pyrimidine-purine-pyrimidine.[21,22] The average stabilizing effect of one ^{m}C replacement has a nominal effect of less than 2 °C.

The influence of Mg^{2+}

Electrostatic screening in the presence of divalent metal ions may relieve repulsion between the charged phosphate groups of the three strands. However, the effect is not equivalent in all recombination-like triplexes. The general trend is that the stabilizing effect is higher in less stable triplex but lower in more stable ones, with one exception, the C3G3A6. We found that the T_{m1}'s are increased by 7.0, 5.3, 5.4, 4.7, 1.9, 1.0, and 1.9 °C in (CT)3A6 (T_{m1} = 36.1 °C), C3A9 (T_{m1} = 40.5 °C), ^{m}C3A9 (T_{m1} = 43.3 °C), C5A7 (T_{m1} = 44.9 °C), (CA)5A2 (T_{m1} = 48.5 °C), (mCA)5A2 (T_{m1} = 53.2 °C), and (CG)3A6 (T_{m1} = 56.3 °C), respectively (Table 3).

DISCUSSION

The formation of recombination-like triplex of all four R-form base triads

The formation of recombination-like triplexes of C3A9, C5A7, and (CA)5A2 which involve only A*(AB T) and C*(C B G) base triads were reported previously.[13] In this paper, we designed triplexes with the third single strand identical, and with parallel orientation, to one of the hairpin duplex stems in order to reveal the contribution of two additional triads, G*(G B C) and T*(T B A). In the mean time, the effect of the sequence dependence can be studied by sequence variations (Table 1). The effect of substitution of cytidines by ^{m}C in the third strand of the oligomer, and the addition of Mg^{2+} ion to the formation of triplex with a recombination-like motif were also reported. All triplex formations are supported by the mixing curves (data not shown) and by the UV thermal melting temperature data.

The relative stabilities of R-form base triads

The melting temperatures of all the studied hairpin double stranded helices from high to low are $5'$-$(CG)_3A_6C_4T_6(CG)_3$ > $5'$-$C_3G_3A_6C_4T_6C_3G_3$ > $5'$-$(CA)_5A_2C_4T_2(TG)_5$ > $5'$-$C_5A_7C_4T_7G_5$ > $5'$-$C_3A_9C_4T_9G_3$ > $5'$-$(CT)_3A_6C_4T_6(AG)_2$ ~ $5'$-$C_3T_3A_6C_4T_6A_3G_3$ (Table 3). In spite of the contribution of the C_4 loop, this order is very close to the ΔG calculated by the nearest neighbor stacking effect shown in the 'Results'.[20] These data are within 1.1 °C to the T_{m2} in the triplex (Table 3). This is within the error of UV melting temperature measurement. The T_{m2} is thereby assigned to the formation of the duplex, and the T_{m1} is the melting temperature of the third strand dissociated from the duplex.

From the summary in Table 3, we can see that the type and number of base triads in the triplex dominate the stability of recombination-like triplex. The order of stability of the base triads is G*(GB C) > C*(CB G) > A*(AB T) > T*(T BA). The postulated hydrogen bonding schemes of these four base triads[12] is shown in Fig. 2. Aside from the fact that the third strand must be parallel to, and be the same as, one strand of the duplex, other criteria are: the bases in the duplex are in Watson-Crick pairing; the third strand is located in the major groove of the duplex; and the bases of the third strand and the bases in duplex are associated with at least two hydrogen bonds. It is interesting to note that G*(G B C) has three hydrogen bonds between the base of the third strand and those in the duplex. Thus, it is the most stable base triad of all. It is also found that the above four base triads have the lowest energies among those in the same category by theoretical calculation.[12] For instance, although T*(T B A) is the least stable among the four base triads, it is still relatively easy to form C*(T B A), G*(T B A), and A*(T B A). Therefore, this can provide some guidance for sequence picking in triplex formation.

The second factor dominating the stability of the triplexes is the sequence of the third-stranded oligomer in our study. Thus, the nearest-neighbor stacking effect will govern the stability when the composition of the base triad is the same. The conclusion can easily be observed as the stabilities of (CG)3A6, (CA)5A2, and (CT)3A6 are larger than those of C3G3A6, C5A7, and C3T3A6, respectively. On the other hand, the T_{m1}'s of (CT)3A6 and (CA)5A2 are still less than those of C5A7 and C3G3A6. This may indicate that the type of base triad plays a major role on the stability.

The ^{m}C modification on the third-stranded oligomer and triplex stability in the various triplex motifs

In the classical pyrimidine-purine-pyrimidine motif, cytidines in the third strand have to be protonated to form a

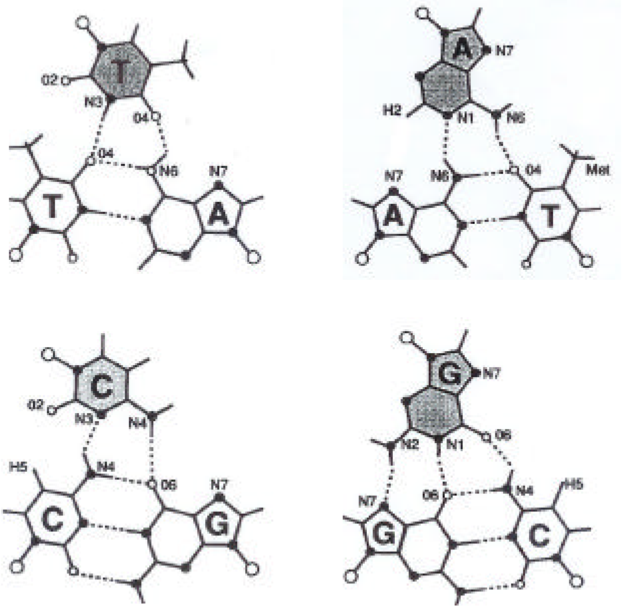

Fig. 2. The postulated hydrogen bonding schemes of R-form triplex. The bases in the third strand are shaded. The possible hydrogen bonds are presented by dotted lines. The N and $C_{1'}$ atoms are denoted by filled and open circles, respectively.

C$^+$*(G B C) triad. The positive charge of the cytidines in the third strand is necessary to enable Hoogsteen base pairing with guanines.[21,22] The stability at neutral pH, however, was realized by modification of the C's to ^{m}C in this study (Table 3). Generally, ^{m}C stabilizes triplex formation in the classical pyrimidine-purine-pyrimidine motif.[21-25] This holds true also for the recombination-like parallel motif studied hitherto. The replacement of three out of five cytidines by ^{m}C in the sequence (CA)5A2, resulting in the single strand 5′-^{m}CACA ^{m}CACA ^{m}CAAA, stabilizes the triplex by 4.7 °C. Similarly, replacing all three cytidines in the single strand C3A9 by ^{m}C, resulting in the single strand 5′-^{m}C ^{m}C ^{m}CAAAAAAAAA, the triplex melting increases by 2.8 °C. The smaller stabilization effect may be explained by the unfavorable juxtaposition of the ^{m}C that was avoided in the first example 5′-^{m}CACA ^{m}CACA ^{m}CAAA. These results are in agreement with the stabilization found in the classical pyrimidine-purine-pyrimidine motif when ^{m}C replaces cytidine.[21-25] Since the T_{m1}'s of ^{m}C3A9 and (^{m}CA)5A2 are lower than those of C5A7 and C3G3A6, respectively, the methylation of cytidine also plays a minor role, compared to the composition of base triad, to the stability of the triplex.

The differential stabilizing effect exerted by the presence of the Mg^{2+} ions

The stabilizing effects of 20 mM of Mg^{2+} ions to (CT)3A6, C3A9, ^{m}C3A9, C5A7, (CA)5A2, (^{m}CA)5A2, C3G3A6, and (CG)3A6 in 200 mM Na$^+$ ions are 7.0, 5.3, 5.4, 4.7, 1.9, 1.0, -0.6, and 1.9 °C (Table 3). The first four are prominent and can easily be detected in UV melting experiments. The results indicated that the stabilizing effect is larger for the less stable triplexes. This is similar to the effect on various DNA structures (i.e., perfect duplex, hairpin duplex, duplex with bulge loop, and duplex containing a mismatched base pair) studied previously in our laboratory.[26] The results showed the Mg^{2+} has the largest stabilizing effect on DNA duplex with mismatched or bulged loop (less stable duplex) and exerted a minimal effect on a hairpin duplex (more stable duplex).[26] Thus, the stabilizing effect of Mg^{2+} may relieve repulsion between the charged phosphate groups on the backbone of the three oligonucleotides in triplex. The Mg^{2+} ions tend to compensate the less stable ordered structures such as duplex and triplex more effectively. It is interesting to note that the Mg^{2+} exerted equal stabilizing effect on the C3A9/^{m}C3A9 and (CA)5A2/(^{m}CA)5A2 pairs. This result indicates that Mg^{2+} affects only the sugar-phosphate backbone but not the bases. Finally, the Mg^{2+} has very little effect on the hairpin duplex portion of the recombination-like triplex (Table 3), congruent with our previous studies.[26] The result may suggest that the formation of recombination-like triplex can further be stabilized by appropriate (such as spermidine in reference 26) cations. Further exploration is worth pursuing.

CONCLUSIONS

We found that two more base triads (T*(T B A) and G*(G B C)) in addition to (A*(A B T)[14] and C*(C B G)[13] can contribute to the formation of recombination-like triplexes. They exhibit major stabilizing effects in triplex formation, and their strengths have been studied systematically and thoroughly. We have provided a systematic approach to examine the contribution of the major factors affecting stability of the triplexes, including: the order of the sequence, the introduction of ^{m}C, and the Mg^{2+} ions. We have provided some guidelines in relaxing the restrictions on triplex formation. The results reported here might have important implications in complicated biological systems where triplex formation is possible.

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REFERENCES

1. Felsenfeld, G.; Miles, H. T. *Annu. Rev. Biochem.* **1967**, *36*, 407.
2. Kan, L. S.; Callahan, D. E.; Trapane, T. L.; Miller, P. S.; Tso, P. O. P.; Huang, D. H. *J. Biomol. Struct. Dynamics.* **1991**, *8*, 911.
3. Cheng, Y. K.; Pettitt, B. M. *Progr .Biophys. Mol. Biol.* **1992**, *58*, 225.
4. Lu, G.; Ferl, R. J. *Int. J. Biochem.* **1993**, *25*, 1529.
5. Radhakrishnan, I.; Patel, D. J. *Structure* **1994**, *2*, 17.
6. Plum, G. E.; Pilch, D. S.; Singleton, S. F.; Breslauer, K. J.

Annu. Rev. Biophys. Biomol. Struc. **1995**, *24*, 319.

7. Frank-Kamenetskii, M. D.; Mirkin, S. M. *Annu. Rev. Biochem.* **1995**, *64*, 65.
8. Praseuth, D.; Guieysse, A. L.; Helene, C. *Biochim. Biophys. Acta.* **1999**, *1489*, 181.
9. Cooney, M.; Czernuszewicz, G.; Postel, E. H.; Flint, S. J.; Hogan, M. E. *Science.* **1988**, *241*, 456.
10. Duval-Valentin, G.; Thuong, N. T.; Hélène, C. *Proc. Natl. Acad. Sci. USA.* **1992**, *89*, 504-508.
11. Ing, N. H.; Beekman, J. M.; Kessler, D. J.; Murphy, M.; Jayaraman, K.; Zendegui, J. G.; Hogan, M. E.; O'Malley, B. W.; Tsai, M. J. *Nucleic Acids Res.* **1993**, *21*, 2789.
12. Zhurkin, V. B.; Raghunathan, G.; Ulyanov, N. B.; Camerini-Otero, R. D.; Jernigan, R. L. *J. Mol. Biol.* **1994**, *239*, 181. Zhurkin, V. B.; Raghunathan, G.; Ulyanov, N. B.; Camerini-Otero, R. D.; Jernigan, R. L. *Structural Biology: State of the Art.* Sarma, R. H.; Sarma, M. H. eds Adenine Press, 1994; pp 43-66.
13. Walter, A.; Schütz, H.; Simon, H.; Birch-Hirschfeld, E. *J. Mol. Recogn.* **2001**, *14*, 122-149.
14. Dagneaux, C.; Gousset, H.; Shchyolkina, A. K.; Ouali, M.; Letellier, R.; Liquier, J.; Florentiev, V. L.; Taillandier, E. *Nucleic Acids Res.* **1996**, *24*, 4506.
15. Shchyolkina, A. K.; Timofeev, E. N.; Lysov, Y. P.; Florentiev, V. L.; Jovin, T. M.; Arndt-Jovin, D. J. *Nucleic Acids Res.* **2001**, *29*, 986.
16. Sun, J. S.; Francois, J. C.; Montenay-Garestier, T.; Saison-Behmoaras, T.; Roig, V.; Thuong, N. T.; Hélène, C. *Proc. Natl. Acad. Sci. USA.* **1989**, *86*, 9198.
17. Collier, D.; Thuong, N. T.; Hélène, C. *J. Am. Chem. Soc.* **1991**, *114*, 1457.
18. Sambrook, J.; Fritsch, E. T.; Maniatis, T. *Molecular Cloning - a Laboratory Manual*, Vol. 2, 2nd edn; Cold Spring Harbor Laboratory Press: New York, 1989, 11.20.
19. Borer, P. N.; Dengler, B.; Tinoco, I.; Jr.; Ullenbeck, O. C. *J. Mol. Biol.* **1974**, *86*, 843.
20. Allawi, H. T.; Santa Lucia, J.; Jr. *Biochemistry,* **1997**, *36*, 10581-10594. Nearest neighbor thermodynamics parameters for internal GA mismatches, *ibid*, **1998**, *37*, 21270.
21. Lin, S. B.; Kao, C. F.; Lee, S. C.; Kan, L. S. *Anti-Cancer Drug Design* **1994**, *9*, 1.
22. Fang, Y.; Wei, Y.; Bai, C.; Tang, Y.; Lin, S. B.; Kan, L. S. *J. Biomol. Str. Dyn.* **1997**, *14*, 485.
23. Singleton, S. F.; Dervan, P. B. *Biochemistry* **1993**, *32*, 14171.
24. Povsic, T. J.; Dervan, P. B. *J. Am. Chem. Soc.* **1989**, *111*, 3059.
25. Fang, Y.; Bai, C. L.; Wei, Y.; Lin, S. B.; Kan, L. S. *J. Biomol. Struct. Dynamics* **1995**, *14*, 471.
26. Hou, M. H.; Lin, S. B.; Yuann, J. M. P.; Lin, W. C.; Wang, A. H. J.; Kan, L. S. *Nucl. Acids Res.* **2001**, *29*, 5121.