Triplex Formation as Functions of Variation of Sequence and Chain Length of Deoxyoligonucleotides at Varied Concentrations of NaCl and $MgCl_2$

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The stabilities of triplexes with two sequences, A_n and $(AG)_n$, were studied by 5′-$C_2A_{16}C_2$/5′-T_n (n = 12, 14, and 16) and 5′-$(AG)_8$/5′-$(CT)_n$ (n = 4, 5, 6, and 7) systems by variation of the chain length (of oligopyrimidine sequences), the composition of base triad (T·A·T versus C^+·G·C), pH (acidic and neutral), and the concentration of NaCl and $MgCl_2$. No stable C^+·G·C base triad was formed at neutral pH. However, C^+·G·C is more stable than T·A·T in acidic conditions. The triplex formed from the 5′-$C_2A_{16}C_2$ and T_{12} mixture with 12 T·A·T units has a melting temperature Tm 11 °C. The triplex formed by 5′-$(AG)_8$/5′-$(CT)_4$ with four T·A·T and four C^+·G·C base triads shows Tm 41 °C. Tm of 5′-$C_2A_{16}C_2$·$[T_{16}]_2$ triplex increased proportionally to the increased NaCl and $MgCl_2$ concentrations. Tm of 5′-$(AG)_8$·$[5'\text{-}(CT)_7]_2$ became constant when NaCl and $MgCl_2$ attained 0.5 M and 5 mM, respectively. The properties of triplexes with A_n and $(AG)_n$ sequences were discussed.

INTRODUCTION

The formation of nucleic acid triplexes, in which the third strand can bind to the major groove of a duplex through interactions of Hoogsteen or reversed Hoogsteen hydrogen bonding, is observed in polyhomonucleotides such as poly(dT) and poly(dA) or poly(U) and poly(A).[1-6] In these cases triplexes with a stoichiometry of two polypyrimidines to one polypurine are formed (Fig. 1). Triplex formation is also found in acidic poly-d(CT) and poly-d(AG) mixtures;[7-11] T and A in this triplex build a T·A·T base triad, and C and G form a C^+·G·C triad (Fig. 1). The structure and base triad conformation have recently been extensively investigated by spectral methods.[10-20]

Sequence-directed triplex formation is recognized of prime importance in biological applications.[6,21-30] In order to search the most favorable conditions and to design the most effective probe for triplex formation, we studied the stability of triplex formation as functions of chain length and base sequence of the oligonucleotide, pH, and concentrations of NaCl and $MgCl_2$ in solution. As shown in Fig. 1, the triplex formed from 5′-$C_2A_{16}C_2$ and T_n (n = 12, 14, and 16) (the top panel of Fig. 1) contains the T·A·T base triad only. The number of homoadenylylic acids remained constant at sixteen. In previous work of this laboratory oligonucleotides with 16 nucleotidylyl units made stable triplexes.[10,11,31] The chain length of homothymidylyl acid (T_n) varied from (n=) 12, 14, and 16, respectively. In order to prevent aggregation of oligo adenylylic acid and oligothymidylic acid, dangling dimeric cytidylyl units were linked to both 3′ and 5′ ends A_{16}.

A triplex containing the C^+·G·C base triad was studied with 5′-$(AG)_8$, a homopurine hexadecanucleotide with alternating adenine (A) and guanine (G) bases in sequence, and 5′-$(CT)_n$ (n = 4, 5, 6, and 7) with alternating cytosine (C) and thymine (T) bases (bottom panel of Fig. 1).[7-11] The chain length of the homopurine strand is maintained at 16 and that of the pyrimidine strand varied from 8 through 10 and 12 to 14. Thus, the longest pyrimidine strand studied was 14, because the third strand (or the Hoogsteen strand) is parallel to the first strand (the homopurine strand). There would be at least one dangling base on the Hoogsteen strand if 16-nucleotidylyl-unit was used.[10,11] No aggregation was observed in the same system in previous studies.[10,11] Both T·A·T and C^+·G·C base triads were formed in the triplex. As no triplex was formed in poly G and poly C in acidic conditions, the involvement of A in sequence is necessary.[32]

EXPERIMENTAL SECTION

Synthesis and Characterization of Oligonucleotides

All oligodeoxyribonucleotides, 5′-$C_2A_{16}C_2$, T_n (n = 12, 14, and 16), 5′-$(AG)_8$, and 5′-$(CT)_n$ (n = 4, 5, 6, and 7) were synthesized on a DNA synthesizer (Applied Biosystem Model 391) using solid-support phosphoramidite chemis-

Dedicated to Professor Sung-Mao Wang (王松茂) on the occasion of his seventieth birthday.

try.[33,34] The oligonucleotide, after being deblocked and cleaved from the solid support, was purified by reverse phase cartridges (Poly-Pak™, Glen Research Corporation) using a procedure recommended by the manufacturers. The chain length and purity of each oligonucleotide were verified with gel electrophoresis.[10,11] The extinction coefficients and hypochromicities of oligodeoxyribonucleotides were determined with enzymatic digestion of snake venom phosphodiesterase I according to the procedure reported by Miller et al.[35] except that 2 mM of $MgCl_2$ and 10 mM of Tris-Cl buffer (pH 8.2) were used in our case.

Thermal Denaturation

UV absorption was measured on Varian DMS 500 and Varian 219 spectrophotometers (Varian Associates, Palo Alto, CA). The dissolved oligomers were 3 μM and 6 μM of homopurine and homopyrimidine oligomers, respectively. The pH of solution was adjusted either 5 with sodium acetate (0.01 M) or 7 with sodium cocadylate (0.01 M). The concentrations of NaCl and $MgCl_2$ of each experiment are stated in the legend of Fig. 2. Thermally induced transitions of each mixture of oligomers was monitored with the latter spectrophotometer having a thermoregulated sample compartment. The sample temperature was controlled with fluid circulating from a temperature-regulated bath monitored with a calibrated thermistor probe inserted in a "dummy" cuvette. Thermal transitions for each mixture were monitored according to UV absorption at 260 nm.[36-38] The total concentrations of mixtures of homopurine and homopyrimidine oligomers were fixed at 9 μM by mixing fractions continuously (for mixing titration experiment).

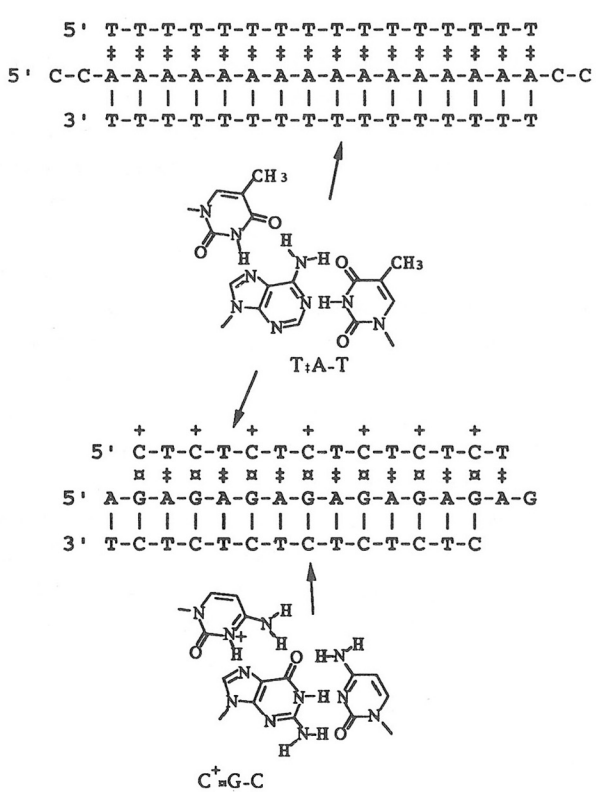

Fig. 1. Schematic drawings of triplexes composed of 5′-$C_2A_{16}C_2$·$[T_{16}]_2$ (top panel) and 5′-$(AG)_8$·$[5'-(CT_7)]_2$ (bottom panel). The Watson-Crick AT/GC hydrogen bondings are represented by "sticks", Hoogsteen TA by •, and C^+G by ¤. The structures of T·A·T and C^+·G·C base triads are shown.

RESULTS AND DISCUSSION

Stability of Triplex Formation versus Chain Length of Oligomers

The UV thermal denaturation profiles of 5′-$C_2A_{16}C_2$ and of T_n (n = 12, 14, and 16) with concentration ratio 1:2

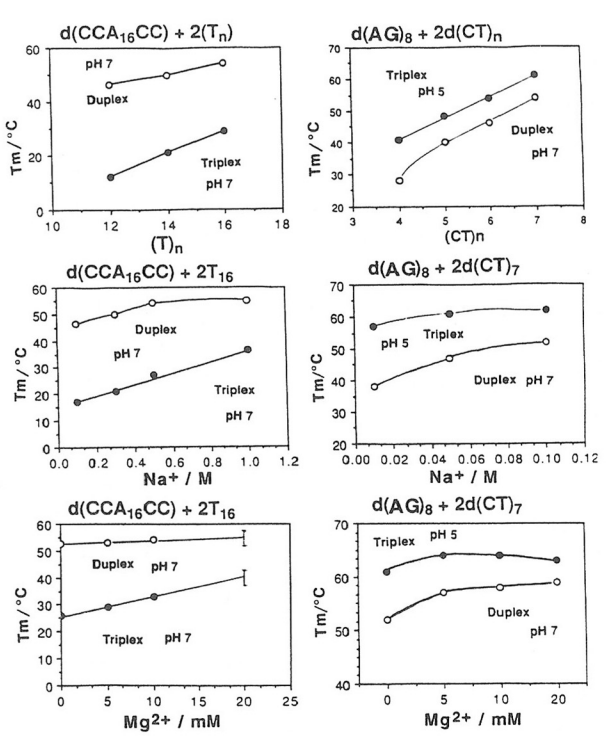

Fig. 2. Profiles of Tm versus chain length of homopyridine strands (top panels), concentration/M of NaCl (middle panels); and the concentraction/mM of $MgCl_2$ (bottom panels). The oligomer systems (5′-$C_2A_{16}C_2$/T_n and 5′-$(AG)_8$/5′-$(CT)_n$), pH, and structures of the complexes (triplex/duplex) are annotated in the figure.

showed two transitions in solution containing NaCl (0.5 M) at pH 7. The smaller one is corresponds to dissociation of the triplex and the other to the duplex. This result is consistent with the mixing titration that had two observed end points, one at 50:50 and the other at 33:67 ratio of 5′-$C_2A_{16}C_2$ and of T_{16} which represented duplex and triplex respectively (data not shown). The melting temperatures (Tm) of both triplex and duplex of this system as a function of chain length of oligothymidylyl acid are shown in the top left panel of Fig. 2. Tm increases as a linear function of the chain length of oligothymidylyl acid from 12 to 16. For the triplex, Tm increases from 12 °C (T_{12}) to 20 °C (T_{14}) and to 28 °C (T_{16}). For the duplex, Tm increases from 47 °C to 50 °C, then to 53 °C for T_{12}, T_{14}, and T_{16}, respectively. The duplex is more stable than the triplex in this system. However, the rate of increase of Tm for a triplex exceeds that of a duplex as a function of chain length of oligothymidylyl acid. These two straight lines in the top panel of Fig. 2 would interact at n = 29 (i.e, T_{29}). We thus predict that the duplex and triplex of 29-mer have the same Tm and that the triplex becomes more stable for a chain greater than 29-mer. This prediction relies on the assumption that Tm is a linear function of chain length. We explain thereby why only a triplex is formed of poly A and poly T (or poly U).[7-9]

The stability of C^+·G·C was tested by a system of 5′-$(AG)_8$ and 5′-$(CT)_n$ (n = 4, 5, 6, and 7) under similar conditions described above except that acetate buffer was used at pH 5. This system forms a triplex only in acidic solution, but a duplex only in neutral conditions.[10,11] Thus, protonation of the cytosine base in the Hoogsteen strand is necessary for the C^+·G·C base triad.

Tm of triplexes of 5′-$(AG)_8$, and 5′-$(CT)_n$ (n = 4, 5, 6, and 7) is a linear function of chain length of the homopyridine strand, shown in the right top panel of Fig. 2. It is interested that the Tm of duplexes, although smaller than those of triplexes, is also a linear function of chain length for n ≥ 5. Tm of 5′-$(AG)_8$-5′-$(CT)_4$ duplex is less than expected perhaps because of a dangling effect of the end bases. The slopes of Tm vs chain length of 5′-$(CT)_n$ for both triplex and duplex are the same. This indicates that the contribution of Hoogsteen C^+·G hydrogen bonding has a similar strength to the Watson-Crick G·C. No interaction is predicted in our tested range of n.

Base Triads C^+·G·C Versus T·A·T

Although the pH values of 5′-$C_2A_{16}C_2$·$[T_n]_2$ and 5′-$(AG)_8$·$[5′\text{-}(CT)_n]_2$ triplex formation differ, the relative strength of C^+·G·C and T·A·T base triads are revealed on comparing Tm in the top panels of Fig. 2. C^+·G·C is obviously more stable base triad than T·A·T. No triplex is formed by deoxyoligonucleotides with a T·A·T base triad of which the chain length is less than 12 in dilute salt conditions. Tm 11 °C of 5′-$C_2A_{16}C_2$·$[T_{12}]_2$ which has 12 T·A·T base triads is less than Tm of 5′-$(AG)_8$·$[(CT)_4]_2$ triplex (41 °C) which contains only eight base triads including four C^+·G·C. This result indicates that C^+·G·C is a more stable base triad than T·A·T. Thus, the stability of triplex with non-consecutive guanine bases in the homopurine sequence is much enhanced with the C^+·G·C base triad. It is interesting to note that no enhancement for the stability of T·A·T base triads resulted from small pH. It is plausible to conclude that the involvement of non-consecutive C^+·G·C base triads may increase the stability of the triplex of all T·A·T base triads. Efforts were made to increase pKa of the C base to bring the stable triplex near neutral pH in several experiments.[28,31,39]

Effect of NaCl and $MgCl_2$

The addition of salt (NaCl and $MgCl_2$) relieves repulsive forces between the negatively charged phosphate groups in the nucleic acid backbone. The secondary structure is stabilized as a result. Triplex systems 5′$C_2A_{16}C_2$ $[T_{16}]_2$ and 5′-$(AG)_8$·$[5′\text{-}(CT)_7]_2$ were chosen as results described in preceding sections were obtained in a solution with 0.5 M of NaCl. These two systems formed a relatively stable triplex in solution with 0.5 M of NaCl. Thus, the chances to form triplexes at a smaller NaCl concentration are high. The results of the effect of NaCl concentration on the stabilities of these two systems are shown in middle panels of Fig. 2. Tm of the 5′-$C_2A_{16}C_2$·$[T_{16}]_2$ triplex showed a linear relationship with NaCl concentration up to 1.0 M. This indicate that this triplex is weakly formed and its secondary structure is stabilized by Na^+. 5′-$(AG)_8$·$[5′(CT)_7]_2$ is a relatively strong triplex with Tm 57 °C in solution with 0.1 M NaCl. The effect of NaCl attained a plateau at 0.5 M of NaCl. This means that the secondary structure of this triplex is orderly already according to its sequence. It is interesting to point out that the salt effect on the corresponding duplexes was just the opposite. The stability of 5′-$(AG)_8$·5′-$(CT)_7$ duplex was less than its triplex. Thus the salt effect attains a maximal level at 0.1 M of NaCl, the largest salt concentration studied (the right middle panel of Fig. 2) and tends to become constant at that point. This duplex is obviously less orderly than its triplex, shown in the figure (right middle). On the other hand, the duplex of 5′-$C_2A_{16}C_2$·T_{16} is more stable than its triplex, shown in the left middle panel of Fig. 2. The salt effect is clearly nearly constant at 0.5 M of NaCl. This result indicates the highly ordered structure of

the duplex.

As Mg^{++} is more effective than Na+ for neutralization, a much smaller concentration (tens of mM) was needed to stabilize the triplex (and duplex) formation. The results are shown in the bottom panel of Fig. 2. Similar to those with NaCl, the triplex of 5′-$C_2A_{16}C_2$·$[T_{16}]_2$ is stabilized linearly with the concentration of $MgCl_2$ up to 20 mM. The effect to 5′-$C_2A_{16}C_2$·T_{16} is minimal. However, Tm of the triplex and duplex of this system are too close (on the UV metlting curve) to be determined accurately at 20 mM of $MgCl_2$ as shown in the left bottom panel of Fig. 2. The interaction of these two lines is estimated at 40 mM according to our extrapolation (data not shown). Thus, a fairly stable (Tm 50 °C) triplex of 5′-$C_2A_{16}C_2$·$[T_{16}]_2$ with all T·A·T base triads was obtained in solution with 40 mM of $MgCl_2$.

$MgCl_2$ exerts only a slight effect on the relatively strong triplex of 5′-$(AG)_8$·[5′-$(CT)_7$]$_2$. Tm becoming constant at 5 mM of $MgCl_2$ was observed for both triplex and duplex (5′-$(AG)_8$·5′-$(CT)_7$). Thus, this triplex is independent of the presence of $MgCl_2$.

Effect of pH

There is no variation of Tm of 5′-$C_2A_{16}C_2$ and T_n (n = 12, 14, and 16) in the pH range 5 - 7 (data are not shown). As no protonation is necessary to form in the T·A·T base triad. The formation of T·A·T is unaffected by pH from 5 to 7. In contrast, protonation on N^3 on cytosine is necessary to form the C^+·G·C base triad. pKa of C bases in oligodeoxynucleotide with alternating C and T in sequence was determined to be about 5.5.[11] Thus, only triplex or duplex formation in system 5′-$(AG)_8$/5′-$(CT)_n$ (n = 4, 5, 6, and 7) occurred at pH 5 or 7, respectively.

CONCLUSION

The stability of triplexes with two the most investigated sequences, A_n and $(AG)_n$, was studied with variation of chain length, composition of base triad, pH, and concentrations of NaCl and $MgCl_2$. As no C^+·G·C base triad is formed at neutral pH, triplexes with the $(AG)_n$ sequence exist only in slight acidic conditions (pH = 5). The shortest triplex formed by 5′-$C_2A_{16}C_2$ and T_n mixture is 12 nucleotidyl units (Tm 11 °C). However, eight nucleotidyl units suffice to form a triplex for the 5′-$(AG)_8$/5′-$(CT)_n$ system (Tm = 41 °C). Thus, C^+·G·C is more stable than T·A·T. Tm of 5′-$C_2A_{16}C_2$·$[T_{16}]_2$ triplex increased proportionally to increase of NaCl and $MgCl_2$ concentrations. However, Tm of 5′-$(AG)_8$·[5′-$(CT)_7$]$_2$ become constant for NaCl and $MgCl_2$ at 0.5 M and 5 mM, respectively. Thus, the properties of triplexes with A_n and $(AG)_n$ sequences are well understood (Fig. 2).

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Key Words

DNA; Triplex; UV; Oligodeoxyribonucleotide.

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