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Silver Ion Unusually Stabilizes the Structure of a Parallel-Motif DNA Triplex

Toshihiro Ihara,*[†,§] Tatsuaki Ishii,[†] Norie Araki,[‡] Anthony W. Wilson,[‡] and Akinori Jyo[†]

Graduate School of Science and Technology, Kumamoto University, 2-39-1 Kurokami,
Kumamoto 860-8555, Japan, Graduate School of Medical Sciences, Kumamoto University,
1-1-1 Honjo, Kumamoto 860-8556, Japan, and PRESTO, Japan Science and Technology Agency,
San-bancho Building, 3-5 Sanbancho, Chiyodaku, Tokyo 332-0012, Japan

Received December 12, 2008; E-mail: toshi@chem.kumamoto-u.ac.jp

The DNA triple helix is one of the most useful recognition motifs in the design of systems for sequence-specific labeling, regulation of gene expression, and construction of DNA-based nanostructures.[1] It can form in two main ways: an oligopyrimidine strand can bind to the major groove of the duplex parallel to the strand carrying the purine tract using Hoogsteen base-pairing, or alternatively, an oligopurine can bind to the purine strand in an antiparallel orientation using reversed Hoogsteen base-pairing. In the parallel motif, cytosines in the third strand need to be protonated at their N3 positions ($pK_a = 4.5$) to form CG.C$^+$ base triplets, which stabilize the triplex by favorable electrostatic effects. Therefore, the stability of triplexes containing CG.C$^+$ largely depends on the pH of the solution. While it is more stable than those consisting entirely of TA.T triplets in weakly acidic solution, its stability is very low under physiological conditions.

Several research groups have devoted their efforts to improving the stability of the triplex at neutral pH.[2] These efforts fall into two categories: (1) addition or covalent attachment of auxiliary molecules that alleviate the electrostatic repulsion between the strands or act as a nonspecific anchor[3] and (2) redesign of the DNA backbone and bases.[4] Here we report an effective alternative method for stabilization of the parallel-motif triple helix of DNA using Ag$^+$ ions. To date, several oligodeoxyribonucleotide (ODN) conjugates carrying metal-chelating moieties have been synthesized.[5] Their binding with the targets was regulated by appropriate metal ions through the specific coordination of the appended chelators. Recently, Ono and co-workers[6] reported that the formation of C−C and T−T mispairings in the duplex are promoted by Ag$^+$ and Hg^{2+}, respectively. In the duplexes, the ions were placed between the bases to form specific bridges (C−Ag$^+$−C, T−Hg^{2+}−T). We report the effect of Ag$^+$ on the stability of triplexes containing CG.C$^+$ base triplets. The silver ion is expected to displace an N3 proton of a cytosine in the CG.C$^+$ base triplet to form a CG.CAg$^+$, as shown in Figure 1A. This would stabilize parallel-motif triplexes even at neutral pH.

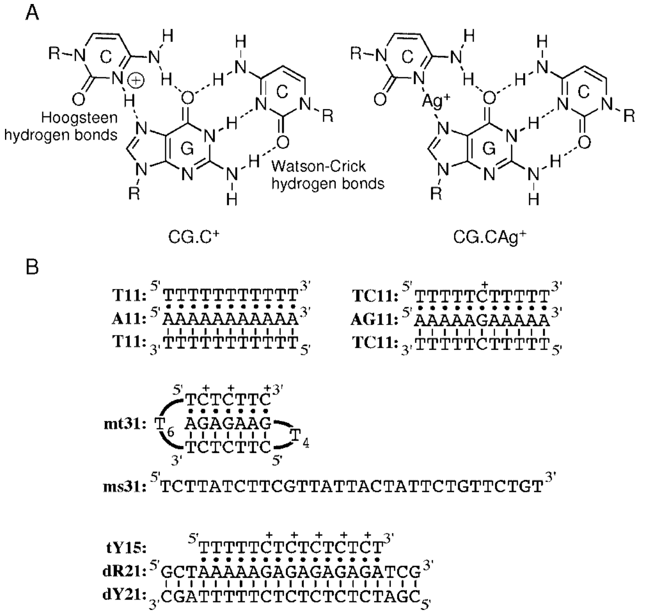

Figure 1. (A) CG.C$^+$ base triplet and the Ag$^+$-mediated base triplet CG.CAg$^+$. (B) Sequences of ODNs used in this study. Bars and dots show Watson−Crick and Hoogsteen base pairings, respectively.

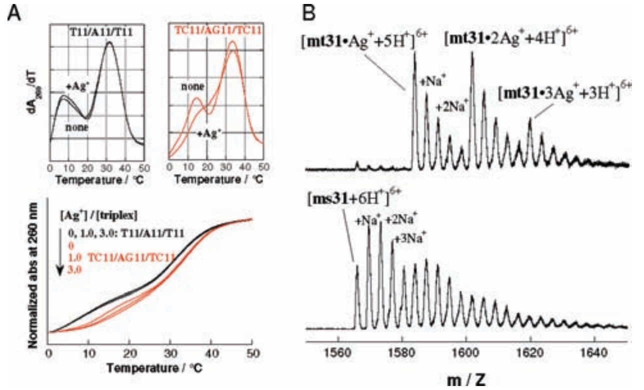

Figure 2. (A) UV melting curves of the triple helixes **T11/A11/T11** (black) and **TC11/AG11/TC11** (red). Each strand (2.5 μM) was dissolved in phosphate buffer (10 mM, pH 8.5) containing 200 mM $NaNO_3$ and 0, 2.5, or 7.5 μM $AgNO_3$. (top) First derivatives of the melting curves obtained in the absence and presence of 7.5 μM Ag$^+$; (bottom) the melting curves. (B) ESI-TOF mass spectra of (top) **mt31** and (bottom) **ms31** in the presence of 1.5 times the amount of Ag$^+$ for possible CG.C$^+$ triplets.

The sequences of ODNs used in this study are shown in Figure 1B. First, UV melting experiments were carried out for the triplexes **T11/A11/T11** and **TC11/AG11/TC11** at pH 8.5 in the absence and the presence of Ag$^+$.[7] **T11/A11/T11** consists of 11 TA.T triplets. Only the central TA.T triplet was displaced by a CG.C$^+$ in **TC11/AG11/TC11**. The melting curves are shown in Figure 2A. The meltings of the triplexes could be resolved in two steps in the absence of Ag$^+$. The transitions at lower and higher temperatures are attributed to triplex−duplex (t−d) and duplex−coil (d−c) transitions, respectively. Upon addition of Ag$^+$ to **TC11/AG11/TC11**, the t−d transition almost disappeared and merged with the d−c transition. Although this effect was already significant when an equimolar amount of Ag$^+$ for the CG.C$^+$ triplet was added, the shape of the curve around the t−d transition showed a further slight change with additional Ag$^+$. On the other hand, the melting curve of **T11/A11/T11** was scarcely changed by Ag$^+$ titration. Silver ions did not affect the d−c transitions for either

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[†] Graduate School of Science and Technology, Kumamoto University.
[‡] Graduate School of Medical Sciences, Kumamoto University.
[§] PRESTO.

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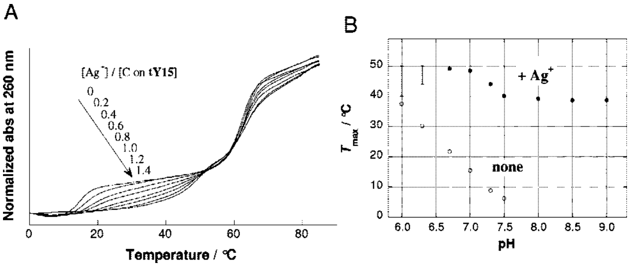

Figure 3. (A) UV melting curves of **dY21/dR21/tY15** obtained in the presence of various amounts of Ag^+. Each strand (2.5 μM) was dissolved in phosphate buffer (10 mM, pH 7.0) containing 200 mM $NaNO_3$ and 0−17.5 μM $AgNO_3$. (B) pH dependence of T_{max} of the t−d transition of **dY21/dR21/tY15** melting in the absence (○) and presence (●) of an equimolar amount of Ag^+ for cytosines in **tY15**.

triplex. Therefore, Ag^+ seems to work only on Hoogsteen hydrogen bonding in the CG.C^+ triplet.

To confirm the specific interaction between CG.C^+ and Ag^+, **mt31**, which forms an intramolecular triplex structure containing three CG.C^+ structures, was subjected to mass spectrometry [(nano)ESI-QqTOF] in the presence of 1.5 times the amount of Ag^+ for the CG.C^+ triplet. The spectra are shown in Figure 2B. Almost all of the **mt31** was detected as Ag adducts with a discrete number of Ag^+ (**mt31**·Ag^+, **mt31**·$2Ag^+$, and **mt31**·$3Ag^+$). On the other hand, the control 31-mer ODN **ms31** with a scrambled sequence was mainly detected as several Na adducts of Ag^+-free forms under exactly the same conditions as for **mt31**. The sequence of **ms31** was designed to not form the intramolecular triplex with CG.C^+ triplets. These results suggest the specific (but probably not very strong) interaction of Ag^+ with CG.C^+ triplets.

The effect of Ag^+ on the stability of the triplex was investigated using the triplex **dY21/dR21/tY15** having multiple CG.C^+ triplets in the sequence. UV melting experiments were performed at pH 7.0 in the presence of various amounts of Ag^+ (Figure 3A). Silver ions raised the temperature of the t−d transition, T_{max}, with significant broadening, and eventually, at the equimolar point with cytosine, a new inflection point appeared at 48.7 °C.[8] The stabilization effect of Ag^+ on the binding of the third strand (**tY15**), ΔT_{max}, was more than 30 °C (48.7−16.8 °C). Meanwhile the d−c transition was scarcely affected by Ag^+ addition in this concentration range. This implies that an Ag^+−triplex complex with a certain structure that has its own quite high thermal stability forms at the equimolar point and that in the complex, Ag^+ only affects Hoogsteen base-pairing of CG.C^+ triplets. The pH effect on T_{max} was monitored in the absence and the presence of Ag^+. Although T_{max} steadily decreased with rising pH in the absence of Ag^+, the T_{max} values observed in the presence of Ag^+ showed a unique biphasic behavior consisting of two pH-independent regions.[9] The slope of the T_{max}-versus-pH plot is related to the number of protons released when the structures melt to give other forms in equilibrium. As we expected, Ag^+ would displace the N3 proton of cytosine in CG.C^+ to form a new triplet, CG.CAg^+, below pH 7. Meanwhile, the proton of the exocyclic amino group of cytosine is considered to be dissociated at pH > 7.0 in the presence of Ag^+.[10] Therefore, another pH-independent equilibrium between CG.C^-Ag^+ and CG + C^-Ag^+ would work in the t−d transition at pH > 8.0. The broadening observed in the transitions before Ag^+ saturation would be derived from the mixture of the triplexes that contain various numbers of Ag^+ on cytosines at different positions, because each of the triplexes should have its own intrinsic thermal stability. Therefore, silver ions are probably incorporated fairly independently into the five binding pockets in **dY21/dR21/tY15** in a saturation process.

To obtain information about the triplex structures, **dY21/dR21/tY15** was subjected to CD measurements [see the Supporting Information (SI)]. While the spectra measured at 7 and 27 °C in the absence of Ag^+ were different, especially in the region of short wavelengths, the corresponding spectra were essentially the same in the presence of Ag^+. This is consistent with the results of the melting experiments shown in Figure 3A. However, the shapes of the CD spectra in the presence of Ag^+ were quite different from the typical spectra of DNA triplexes. The coordination distance in N−Ag^+−N would be longer than that of the Hoogsteen hydrogen bonds in CG.C^+. Model studies show that the cytosines on the third strand are forced to be twisted from the plane of Watson−Crick GC pairs in CG.CAg^+ triplets (see the SI). This nonplanarity of five CG.CAg^+ triplets in **dY21/dR21/tY15** seems to unusually alter the whole structure of the triple helix.

We have shown that Ag^+ remarkably stabilizes the structure of a parallel-motif DNA triplex. Several ligands that specifically bind with DNA triplexes have been developed.[3a,b] The stabilization effect of Ag^+ favorably compares with those for these ligands. This very simple method would be a good choice for the stabilization of DNA triplexes, especially in vitro.

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Supporting Information Available: Additional melting curves and CD spectra of **dY21/dR21/tY15** and the results of DFT calculations for the Ag^+-mediated base triplets. This material is available free of charge via the Internet at http://pubs.acs.org

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(7) We first attempted the melting experiments at pH 7. However, the triplex **TC11/AG11/TC11** melted almost cooperatively. To evaluate both transitions (t−d and d−c) separately, the pH was raised to 8.5.

(8) The maximum temperatures of the first derivative of the melting curves, T_{max}, were used as the indicators of triplex stabilities.

(9) T_{max} values around pH 6 were not precisely determined because the t−d transitions became broad, as in the melting curves before Ag^+ saturation measured at pH 7 (Figure 3A). However, the curves became sharper upon further addition of Ag^+ (2 times for C). This supports the idea of proton displacement by Ag^+ in the CG.C^+ base triplet.

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