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CRITICAL REVIEW

Binding of metal ions by pyrimidine base pairs in DNA duplexes†

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Pyrimidine base pairs in DNA duplexes selectively capture metal ions to form metal ion-mediated base pairs, which can be evaluated by thermal denaturation, isothermal titration calorimetry, and nuclear magnetic resonance spectroscopy. In this *critical review*, we discuss the metal ion binding of pyrimidine bases (thymine, cytosine, 4-thiothymine, 2-thiothymine, 5-fluorouracil) in DNA duplexes. Thymine–thymine (T–T) and cytosine–cytosine (C–C) base pairs selectively capture Hg(II) and Ag(I) ions, respectively, and the metallo-base pairs, T-Hg(II)-T and C-Ag(I)-C, are formed in DNA duplexes. The metal ion binding properties of the pyrimidine–pyrimidine pairs can be changed by small chemical modifications. The binding selectivity of a metal ion to a 5-fluorouracil–5-fluorouracil pair in a DNA duplex can be switched by changing the pH of the solution. Two silver ions bind to each thiopyrimidine–thiopyrimidine pair in the duplexes, and the duplexes are largely stabilized. Oligonucleotides containing these bases are commercially available and can readily be applied in many scientific fields (86 references).

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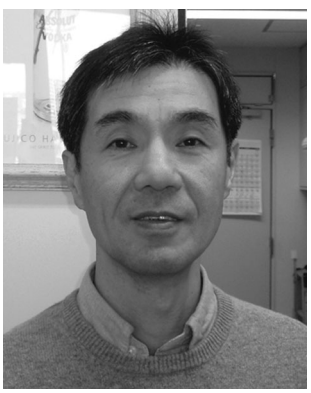
1. Introduction

Much research directed towards extending the functionality of base pairs in DNA duplexes with respect to artificial gene control, as well as the development of DNA structure-based functionalized biopolymers, has been performed.[1–9] Synthetic oligodeoxyribonucleotides (ODNs) containing artificial bases have been used to form metal-mediated base pairs (metallo-base pairs), in which the hydrogen bonds in Watson–Crick (W–C)-type base pairs, as found in natural DNA,



Akira Ono

Akira Ono was born in 1957 in Saijo-shi, Ehime-ken, Japan. He received his BSc degree in 1981, MSc degree in 1983, and PhD in 1986 from the Faculty of Pharmaceutical Sciences, Hokkaido University under the supervision of Professor Tohru Ueda. Afterward, he joined the Faculty of Pharmaceutical Sciences, Hokkaido University as an assistant professor in Professor T. Ueda's group. In 1993, he moved to the Graduate School of Sciences, Tokyo Metropolitan University as an Associate Professor. In 2005, he moved to Department of Material & Life Chemistry, Faculty of Engineering, Kanagawa University as a Professor. His current research interests are synthesis of bioactive nucleic acids, nucleic acids–metal interactions, and development of nucleic acids based functional materials.

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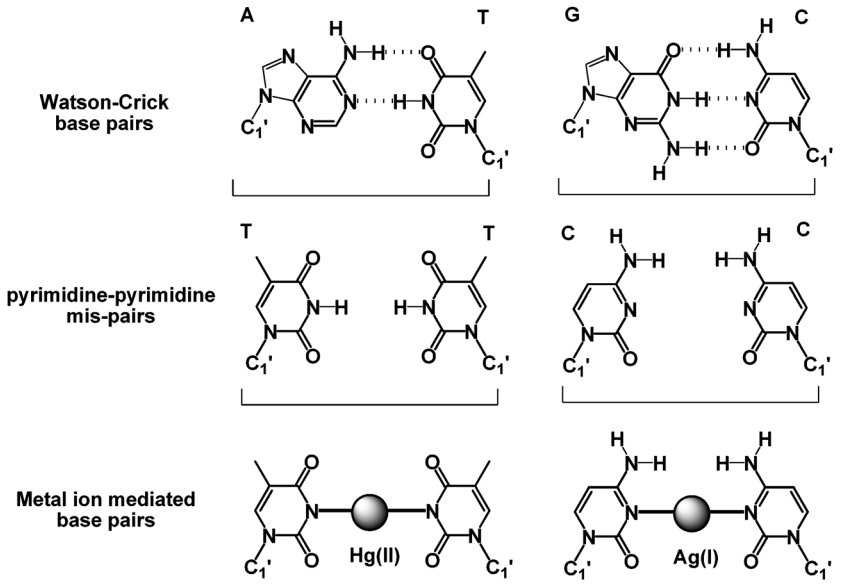
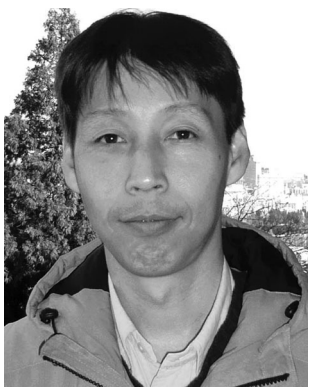


Fig. 1 Schematic representations for W–C base pairs, pyrimidine–pyrimidine mis-pairs, and metal ion-mediated base pairs (metallo-base pairs).

are replaced by metal–base bonds.[1–9] Alternative methods for generating metal-mediated base pairs in DNA duplexes using only naturally occurring pyrimidine bases have also been reported.[10–21] Thymine–thymine (T–T) and cytosine–cytosine (C–C) pairs selectively capture Hg(II) and Ag(I) ions, respectively, and the metallo-base pairs, T-Hg(II)-T and C-Ag(I)-C, are formed in DNA duplexes. In addition to pyrimidine-metal-pyrimidine pairs in DNA duplexes, the formation of RNA duplexes containing U-Hg(II)-U and C-Ag(I)-C pairs, and the formation of a DNA triplex containing a C-Ag(I)-G-C triad have been reported.[22–24]

As mercury–(T–T) and silver–(C–C) binding is highly selective, novel DNA structure-based sensors capable of selectively detecting Hg(II) and Ag(I) ions in aqueous solutions,[14,16,25,26] the redox-state of these solutions,[15] and single nucleotide polymorphisms[18–20] have been developed. Attempts to enzymatically incorporate the metal ion-mediated base pairs have been reported,[27,28] as has a DNA-based nanomachine, which is activated by Hg(II) ions.[29] DNA-Ag(I) ion binding has been used to form silver nanoclusters.[30,31] Synthetic polymers carrying thymine bases have the potential to act as Hg(II) ion adsorbents.[32]



Yoshiyuki Tanaka

Yoshiyuki Tanaka was born in 1967 in Hiroshima. He received his BSc degree in 1991 and MSc degree in 1993 from the Faculty/Graduate School of Pharmaceutical Sciences, Osaka University under the supervision of Professor Ken-ichi Tomita, and received Ph.D. in 1998 from the Graduate School of Sciences, Osaka University under the supervision of Professor Yoshimasa Kyogoku. Afterward, He joined AIST at Tsukuba as a post-doctoral fellow under the direction of Professor Kazunari Taira. In 2001, he moved to the Graduate School of Pharmaceutical Sciences, Tohoku University as a Research Instructor. Now he is an Associate Professor at the Graduate School of Pharmaceutical Sciences, Tohoku University. He has belonged to laboratory of molecular transformation (Professor Yoshinori Kondo's laboratory) since he came to Tohoku University. His current research interests are not only nucleic acids–metal interactions, but also mechanistic studies on functional RNA/DNA molecules.

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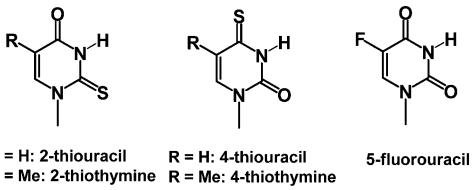

Fig. 2 Structure of the minor bases: 2-thiopyrimidines, 4-thiopyrimidines, and 5-fluorouracil.

Recently, many studies have focused on the experimental and theoretical investigation of the conductive nature of metallo-base pairs.[33–40] Consequently, there is a great deal of interest in methods for capturing desired metal ions in DNA strands, because they are important for the development of new materials, such as DNA wires containing metal ions, new sensors capable of detecting various metal ions in aqueous solutions, and nanomaterials containing metals. One advantage of using natural pyrimidine bases to capture the metal ions is that these studies can be carried out using only commercially available oligonucleotides, and such experiments can be reproduced in any laboratory. Many of the oligonucleotides introduced in this article, except the isotopically labelled oligonucleotides, can be purchased or synthesized using commercially available monomer units.

From synthesized ODNs, of which the sequences are precisely controlled, DNA duplexes containing pyrimidine–pyrimidine pairs at desired sites can be prepared. Because pyrimidine bases are smaller than purine bases, and positions of the bases are fixed to the sugar-phosphate backbones, there should be some regions between bases in pyrimidine–pyrimidine pairs into which metal ions can be incorporated (Fig. 1). Hg(II) and Ag(I) ions can be incorporated between T–T and C–C pairs, respectively, and form T-Hg(II)-T and C-Ag(I)-C pairs.

Minor bases (2-thiouracil and 4-thiouracil) are observed in RNA within cells (Fig. 2).[41] The anticancer drug 5-fluorouracil is incorporated into RNA strands after several biological processes.[42] ODNs containing these bases can be purchased or readily synthesized using commercially available amidite units.

In this article, methods for investigating both the formation and structures of metallo-base pairs are outlined.[43] The thermal denaturation method[44,45] has been widely used to examine thermal stabilities of nucleic acid structures, and has been applied to identify metal ions bound tightly to base pairs in DNA duplexes. Isothermal titration calorimetry (ITC), an approved method for thermodynamically analyzing bio-molecular complex formation,[46] has been efficiently used for thermodynamically analyzing metal ion–base pair binding. Nuclear magnetic resonance (NMR) spectroscopy, widely used for determining structures and behaviours of biopolymers, such as proteins and nucleic acids in solution,[47] has been used to obtain structural information regarding metal ion-mediated base pairs in solution.

1.1 Thermal denaturation, isothermal titration calorimetry, and NMR

The thermal denaturation method has been used to identify metal ions bound tightly to base pairs in duplexes.[10,16] The absorbance at approximately 260 nm of a solution containing

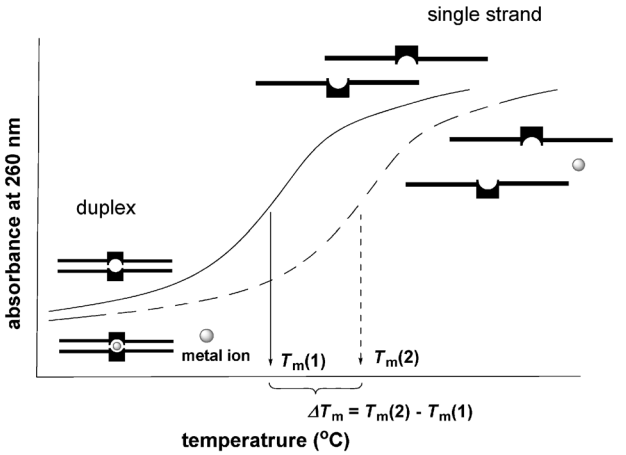

Fig. 3 Schematic representation of the thermal denaturation profiles for a duplex and a duplex containing a metal ion-mediated base pair.

a duplex is monitored as the solution is gradually heated. At lower temperatures, a duplex structure is maintained. As the solution temperature is increased, the duplex dissociates and is ultimately entirely single-stranded (Fig. 3; solid curve). A transition curve is observed between the temperature at which the duplex begins to dissociate and the temperature at which the duplex is completely dissociated. Within this temperature range, the DNA strands are at equilibrium between the duplex and single-stranded states. As the temperature is increased further, the equilibrium shifts to the single-stranded state. The melting point (T_m) is the temperature at which the concentration of the duplex equals the concentration of single-stranded DNA: [duplex] = [single strand].[44,45]

When a metal ion binds tightly to a base pair, and the resulting metallo-base pair is sufficiently stable, the entire duplex structure becomes more thermally stable than the metal-free duplex and the transition curve shifts to a higher temperature region (Fig. 3; dotted line). T_m values are used for comparing the stabilities of duplexes; a duplex with a higher T_m value is generally considered more stable than a duplex with a lower T_m value. The difference in T_m values (ΔT_m) corresponds to a difference in stabilities between duplexes in the presence or absence of metal ions. Thermal denaturation methods detect only tightly bound metals, and this method ignores background nonspecific metal ion–duplex binding. Metal ions are known to bind to various sites on nucleotides, such as bases and phosphates,[41,48] which are sometimes detected as background noise by more sensitive methods, such as ESI-MS and NMR.

As shown schematically in Fig. 4, the decomposition of a whole metal–duplex complex can be observed by thermal denaturation. However, it is difficult to precisely and independently observe the decomposition of metallo-base pairs and conformational alteration and unwinding of the duplex structure.

Isothermal titration calorimetry (ITC) has been used effectively for thermodynamically analyzing metal ion–base pair binding.[13,17–21] Since the solution is held near room temperature, a duplex structure is maintained throughout the measurement and the thermodynamic parameters for the

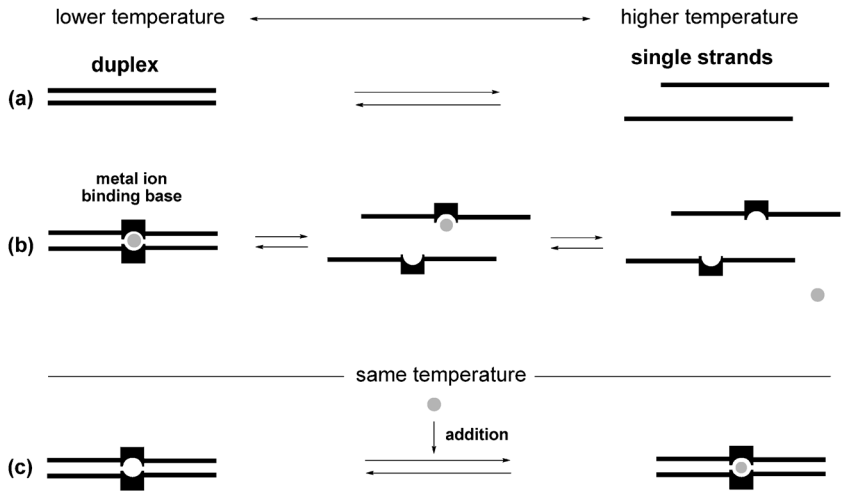

Fig. 4 (a) Dissociation process of an unmodified duplex is regarded as the equilibrium between the duplex and single-strand states. (b) Dissociation of a duplex containing a metal ion-mediated base pair contains multiple intermediates. (c) A metal ion–base pair binding process observed directly.

binding of a metal ion and a base pair can be directly observed (Fig. 4c). Thermodynamic parameters provide information about the binding process, such as the effect of hydration states.

Heteronuclear multidimensional NMR spectroscopy, widely used for determining the structures and behaviors of biopolymers, such as proteins and nucleic acids in solution,[47] has also been used to study metal ion–base pair binding. Chemical and biological methods for preparing stable isotopically labeled DNA and RNA fragments have been developed, and isotope-enriched short oligonucleotides have been used in NMR measurements.[49–51] NMR studies of metallo-base pairs have been described in recent review articles.[52,53]

2. Metal ion-mediated, natural pyrimidine base pairs, T-Hg(II)-T and C-Ag(I)-C

We describe the structure and thermodynamics of T-Hg(II)-T and C-Ag(I)-C pairs, effects of W–C pairs neighboring the metallo-base pairs, and the strand arrangements of duplexes containing the metallo-base pairs.

2.1 T-Hg(II)-T and C-Ag(I)-C complexes

Hg(II) and Ag(I) ions are known to preferentially bind nucleobases.[41,48] Due to the high toxicity of mercury,[54] interactions between mercury ions and nucleic acids have been widely investigated. Binding of Hg(II) ions to large DNA and RNA fragments, which causes structural denaturation and destabilization, has been studied using pH titration, UV absorption, and CD.[55–59] Prof. Katz proposed a binding scheme in 1963 (T-Hg(II)-T), which is shown in Fig. 1.[56] A crystal structure revealing a 2 : 1 complex consisting of 1-methylthymine and Hg(II) ions was reported,[60] corroborating the binding scheme. The binding of Hg(II) ions with a large DNA fragment triggers the dissociation of A–T pairs, followed by the formation of T-Hg(II)-T. The formation of T-Hg(II)-T pairs within cells could be a bioprocess involved in mercury cytotoxicity.[54]

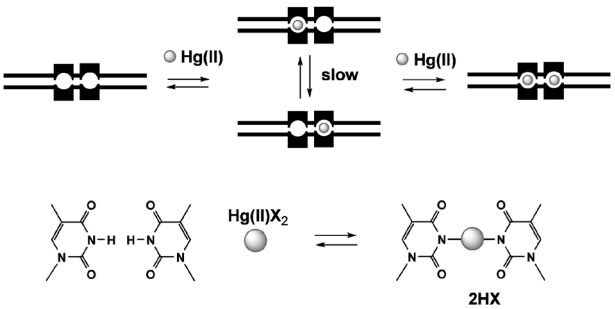

Fig. 5 Schematic representation of the Hg(II) titration process.

Duplexes having T–T pairs can be prepared from synthesized ODNs, of which the sequences are precisely controlled. Using duplexes containing T–T pairs, the binding process of Hg(II) ions and T–T pairs within the T-Hg(II)-T region can be investigated directly.[10,11,61] Kuklenyik and Marzilli demonstrated that mercury-mediated thymine pairs could be formed within duplexes.[61] T–T pairs in duplexes specifically capture Hg(II) ions to form highly stabilizing T-Hg(II)-T pairs.[10] According to Hg(II) ion binding, the imino protons of the thymines dissociate (Fig. 5), which was confirmed by the disappearance of signals from the imino protons in the ^{1}H-NMR spectra.[10,61] In our study, a duplex containing two consecutive T–T pairs was used for NMR studies.[10,11] In a titration study, it was found that once a Hg(II) ion binds to a T–T pair, the Hg(II) ion remains for a long time (on the NMR time scale) and does not readily transfer to a neighboring T–T pair, indicating the tightness of Hg(II) ion binding.

Recently, ^{15}N-NMR has been used to study hydrogen-bond formation and the metallation of nucleobases in ^{15}N-enriched DNA and RNA.[50,51] To demonstrate formation of a specific hydrogen bond, we measured the *J*-coupling constant across the assumed hydrogen bond (Fig. 6a).[62,63] Analogously, if N3–Hg(II) bonds are formed in the T-Hg(II)-T pair, the observation of an ^{15}N–^{15}N *J*-coupling across Hg(II) would be evidence supporting the formation of the T-Hg(II)-T pair

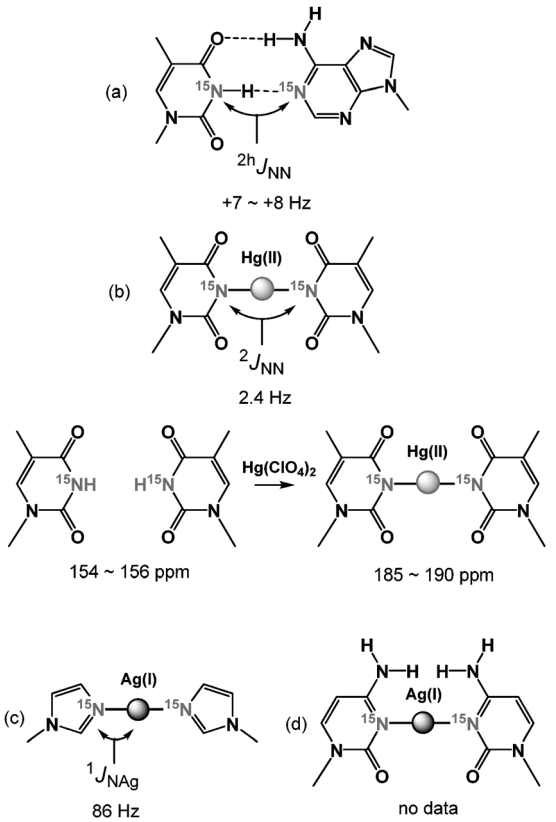

Fig. 6 (a) *J*-coupling across a hydrogen bond (ref. 62 and 63). (b) $^{2}J_{NN}$ and chemical shifts of T-Hg(II)-T pairs (ref. 12 and its supporting information). (c) $^{1}J_{NAg}$ of imidazole-Ag(I)-imidazole pair (ref. 70). (d) Predicted structure of C-Ag(I)-C pair.

(Fig. 6b).[12] The observed $^{2}J_{NN}$ value (2.4 Hz),[12] demonstrating the formation of N–Hg–N bonds, was similar to the theoretical prediction.[64]

Recently, formation of N–Ag bonds was confirmed by a ^{15}N-NMR study, in which ^{15}N-labeled imidazole was incorporated into ODNs and used as metal ion-binding sites.[65] In the experiment, a $^{1}J_{NAg}$ coupling constant was observed, also supporting N–Ag bond formation in the duplex (Fig. 6c).

Using a thermal denaturation study, it was found that duplexes containing C–C pairs were stabilized significantly in the presence of Ag(I) ions, suggesting that silver ion-mediated base pairs (C-Ag(I)-C) were formed.[16] A stoichiometry of 1 : 1 (Ag(I) ion to C–C pair) was confirmed using thermal denaturation, ^{1}H-NMR, ITC, and ESI-MS.[16–21] We have proposed a binding scheme for C-Ag(I)-C (Fig. 6d), but we lack experimental data confirming the actual structure. A possible method to obtain experimental evidence to corroborate the silver ion binding position is ^{15}N-NMR spectroscopy; however, we have not yet obtained results that describe the binding mechanism.

ITC studies indicated that T-Hg(II)-T and C-Ag(I)-C formation is enhanced by both negative enthalpic and positive entropic changes.[13,17–21] As described above, N3–Hg bonds in the T-Hg(II)-T pairs have covalent characteristics and formation of the bonds should facilitate T-Hg(II)-T formation through negative enthalpy changes. The structure of metallo-base pairs in a duplex suggests that the mercury atom in the

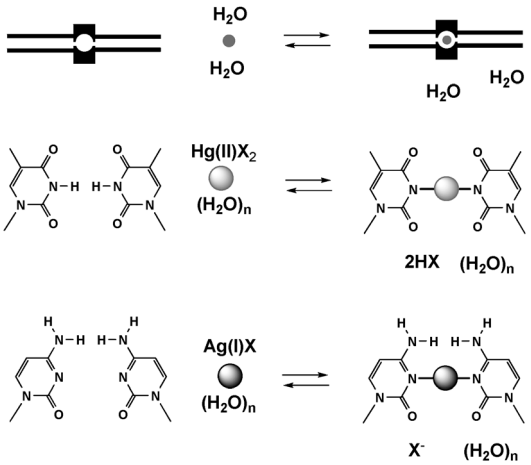

Fig. 7 Schematic representation of the formation of metal ion-mediated base pairs with dehydration of the metal ions.

T-Hg(II)-T pair is sheltered from water molecules.[66] Thus, water molecules assembled around the Hg(II) ion[67] become free with the insertion of the Hg(II) ion into the T–T pair, which should accelerate T-Hg(II)-T formation through positive entropy changes *via* dehydration[66] (Fig. 7). The thermodynamics of C-Ag(I)-C formation could be described similarly, and a binding scheme in which a Ag(I) ion binds between N3 positions has been proposed.[16] However, as mentioned above, we have not yet obtained results that describe the binding scheme.

2.2 Effect of neighboring W–C pairs

A metal ion-binding pocket is formed from a mismatched pair and neighboring W–C pairs, and thus stabilities of the metallo-base pairs depend on neighboring sequences. An example is shown in Table 1.[68] Stabilities of metallo-base pairs varied, depending on the neighboring W–C pairs. For example, the Hg(II) ion binding site in the sequence 5′-GTC-3′–3′-CTG-5′ (entry 5) was obviously more stable than that in the sequence 5′-CTG-3′–3′-GTC-5′ (entry 6). Also, there is a difference in thermal stability between Ag(I) binding sites in the same neighboring W–C pairs, 5′-GCC-3′–3′-CCG-5′ (entry 11), and 5′-CCG-3′–3′-GCC-5′ (entry 12). Though the metallo-base pairs were surrounded by the same G–C pairs, the stabilities of the duplexes differed greatly because the order of the C and G residues was reversed, 5′-GTC-3′ to 5′-CTG-3′. The metal ion could contact and bond with atoms in the neighboring W–C pairs, and such a coordination pattern could be precisely detected by X-ray crystallographic analyses.[43]

2.3 Strand arrangement

Strand arrangement is important for discussing the structure and function of metal ion arrays formed along duplexes. In natural DNA duplexes, the DNA strands run in opposite directions, and in such cases the orientation of the duplex strands is defined as anti-parallel.[41] As discussed below, in duplexes containing (or consisting of) metallo-base pairs, strand arrangements can be both anti-parallel and parallel, depending on the properties of metallo-base pairs (Fig. 8).

Table 1 T_m values of duplexes in the presence (+M) and absence (−M) of metal ions. Each solution contained 2 μM of the oligomer in 10 mM Mops, 100 mM $NaNO_3$, pH 7.1, in the presence of $Hg(ClO_4)_2$ (4 μM) for entries 1–6, and in the presence of $AgNO_3$ (4 μM) for entries 7–12. The T_m values were averages of two measurements

5′-GTGACCNNNGCAGTG-3′
3′-CACTGGNNNCGTCAC-5′

	NNN	T_m (+M)	T_m (−M)	ΔT_m
1	5′-**ATA**-3′ 3′-**TTT**-5′	56	47	9
2	5′-**ATT**-3′ 3′-**TTA**-5′	57	49	8
3	5′-**TTA**-3′ 3′-**ATT**-5′	53	48	6
4	5′-**GTG**-3′ 3′-**CTC**-5′	60	55	5
5	5′-**GTC**-3′ 3′-**CTG**-5′	64	55	9
6	5′-**CTG**-3′ 3′-**GTC**-5′	59	56	3
7	5′-**ACA**-3′ 3′-**TCT**-5′	51	43	8
8	5′-**ACT**-3′ 3′-**TCA**-5′	52	43	9
9	5′-**TCA**-3′ 3′-**ACT**-5′	52	45	7
10	5′-**GCG**-3′ 3′-**CCC**-5′	56	52	4
11	5′-**GCC**-3′ 3′-**CCG**-5′	61	54	7
12	5′-**CCG**-3′ 3′-**GCC**-5′	53	51	2

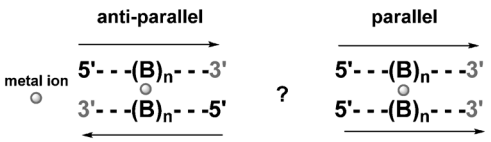

Fig. 8 Schematic representation of the strand arrangements of duplexes containing metallo-base pairs.

In anti-parallel duplexes, A–T and G–C base pairs, and also the metallo-base pairs T-Hg(II)-T and C-Ag(I)-C, are in Watson–Crick (W–C) geometry (Fig. 9). It has been reported that parallel duplexes can be formed with significant stability if the strand orientation is fixed in parallel using a hairpin structure.[69] In the parallel duplexes, A–T pairs are formed in reverse W–C geometry (Fig. 9), but G–C pairs in reverse W–C geometry are unstable. We report the formation of the metallo-base pairs, T-Hg(II)-T and C-Ag(I)-C, in parallel duplexes of which strand orientations were fixed by covalently linking bases at the duplex ends (Fig. 9).[70] In this case, the metal ion-mediated base pairs are in reverse W–C geometry.

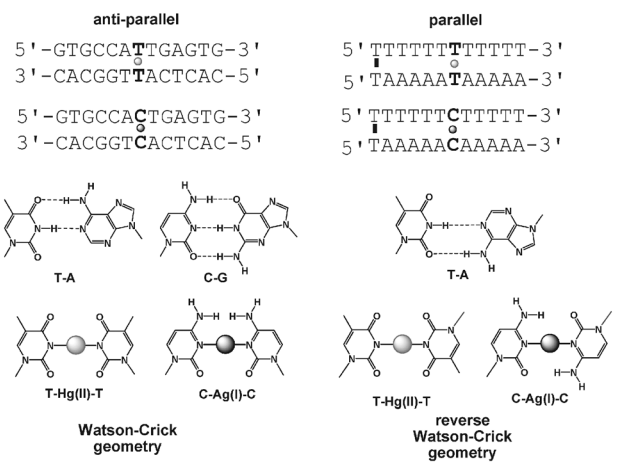

Fig. 9 Base pairs in anti-parallel and parallel duplexes.

When mixed, homothymidylic acid (T_n) and homodeoxyadenylic acid (dA_n) intrinsically form antiparallel and parallel duplexes. Because anti-parallel duplexes are more stable than parallel duplexes, dA_n and T_n form anti-parallel duplexes under physiological conditions. However, if the metallo-base pairs are more stable in reverse W–C geometry than in W–C geometry, duplexes containing the metallo-base pairs should preferentially form in the parallel orientation.

Based on the structure of the T-Hg(II)-T pair, the metallo-base pair has similar stability in both W–C and reverse W–C geometries (Fig. 10a). Megger and Müller[71] suggested that oligodeoxycytidylic acid can form a Ag(I) ion-mediated parallel duplex structure, based on CD spectra of the oligomer in the presence of Ag(I) ions (Fig. 10b). Recently, Urata and co-workers[72] reported that a duplex containing a 5-methyl-isocytosine–cytosine pair was able to capture a Ag(I) ion, and that the duplex containing the metallo-base pair was highly stable (Fig. 10c). The arrangement of atoms around the silver ion in 5-methylisocytosine-Ag(I)-cytosine is similar to that in C-Ag(I)-C with reverse W–C geometry, which may also support the hypothesis that C-Ag(I)-C is more stable in a reverse W–C geometry.

We expect that duplexes consisting of T-Hg(II)-T pairs, $[T\text{-}Hg(II)\text{-}T]_n$, may be similarly stable in both anti-parallel and parallel arrangements (Fig. 10a). Currently, we cannot determine which arrangement is more stable. Duplexes consisting of C-Ag(I)-A pairs, $[C\text{-}Ag(I)\text{-}C]_n$, may favor parallel orientations. It is known that, in appropriate solutions, homocytidylic acids form parallel-stranded double helical structures in which C–C pairs are formed in reverse W–C geometry,[73] and deoxycytidylic acids form the four stranded structure (the *i*-motif), in which two parallel-stranded helices are combined.[74] In both cases, cytosine bases are protonated and three hydrogen bonds are formed in a C–C pair in reverse W–C geometry (Fig. 10d). The insertion of a Ag(I) ion between the cytosine bases may function in a manner similar to that of a proton.

One of the difficulties in preparing long duplexes of metallo-base pairs is the formation of undesirable complexes containing hairpin-like motifs (Fig. 11). Polonius and Müller[75] proposed a method for preparing a long metal ion array with a duplex structure containing artificial base residues. They observed the formation of the Ag(I) ion-mediated base pair, 1-deazaadenine-Ag(I)-thymine, in a Hoogsteen-type geometry, which may result in parallel-stranded, double-helical structures.[76] The 1-deazaadenine-Ag(I)-thymine pair was much more stable than the other possible metallo-base pairs, 1-deazaadenine-Ag(I)-1-deazaadenine and T-Ag(I)-T, and the desired parallel duplex consisting of 1-deazaadenine-Ag(I)-thymine pairs was obtained. Here, we suggest an alternative strategy for preparing long duplexes consisting of metallo-base pairs. In cases where

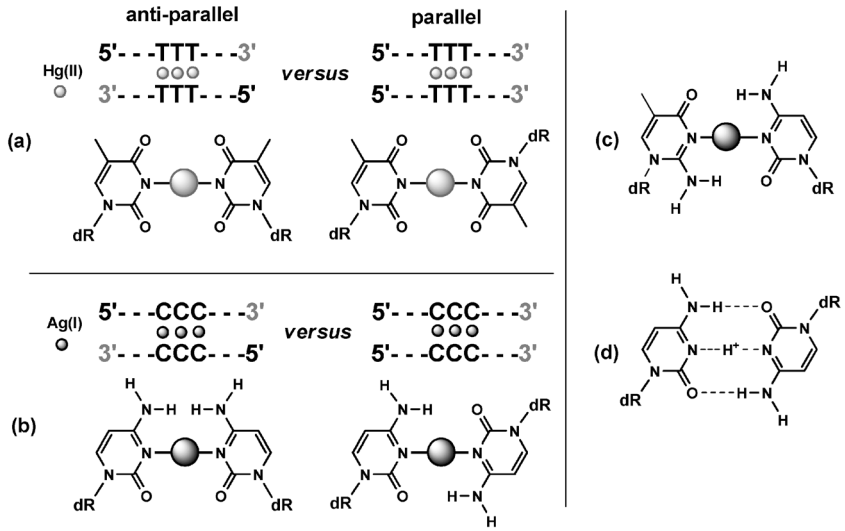

Fig. 10 Schematic representations of metallo-base pair formations in anti-parallel and parallel orientations.

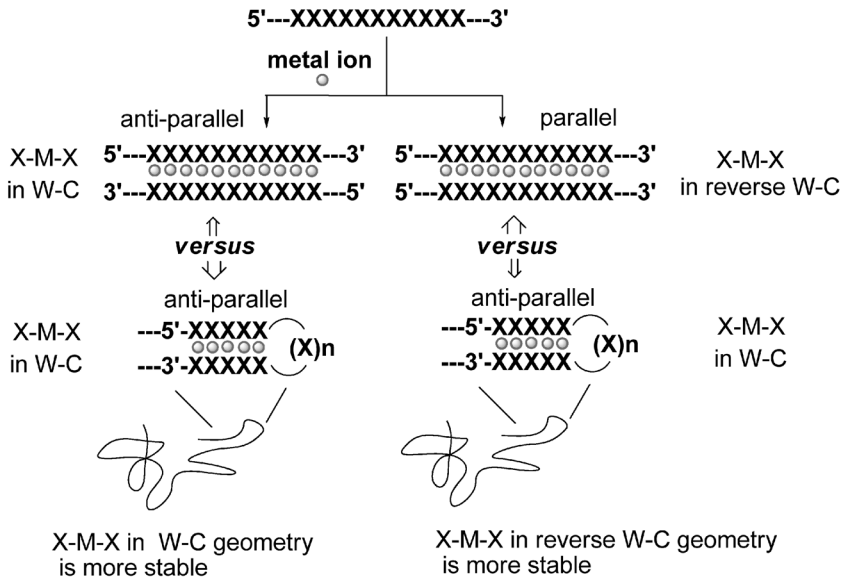

Fig. 11 Schematic representation of possible anti-parallel and parallel duplexes and complexes containing hairpin motifs.

the reverse W–C geometry is much more stable than the W–C geometry, long duplexes will be preferentially formed. As discussed above, C-Ag(I)-C pairs in reverse W–C geometry are more stable than those in a W–C geometry. Thus, by choosing appropriate conditions, polymeric duplexes consisting of C-Ag(I)-C pairs could be prepared by mixing polycytidylic acids (or polydeoxycytidylic acid) and Ag(I) ions.

3 Other pyrimidine–pyrimidine base pairs

In this section, metal ion binding properties of pyrimidine–pyrimidine base pairs in duplexes, excluding typical T-Hg(II)-T and C-Ag(I)-C pairs, are described.

3.1 T-Hg(II)-C and T-Ag(I)-C pairs

Recently, Urata and co-workers reported that a duplex containing a T–C pair was moderately stabilized in the presence of Ag(I) ions, possibly because a T-Ag(I)-C pair was formed.[72] Independently, we observed that a duplex containing a T–C pair was moderately stabilized in the presence of both of Ag(I) and Hg(II) ions. In Fig. 12, thermal denaturation profiles of a duplex containing a T–C pair in the presence of Hg(II) ions and Ag(I) ions are shown.[77] In this experiment, the duplex was stabilized largely in the presence of Hg(II) ions. Alternatively, the stabilizing effect of Ag(I) ions was less than that of Hg(II) ions. Direct comparison of our work with Urata's results is not revealing because the stabilities of the metal–base pairs vary, depending on the neighboring W–C base pairs, as mentioned in Section 2.2.

The predicted structures of the metallo-base pairs are shown in Fig. 12. The imino proton of the thymine residue, replaced by a Ag(I) ion, combined with the cytosine residue, coordinates the silver ion. Thus, T-Ag(I)-C is neutral in such a binding scheme. A similar binding scheme was suggested for

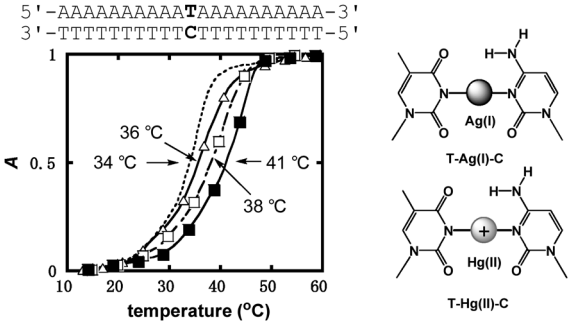

Fig. 12 Left: relative absorbance, $A = [(A_{t°C} - A_{10°C})/(A_{60°C} - A_{10°C})]$ at 260 nm *versus* temperature for the mixtures: 5′-d$(A)_{10}T(A)_{10}$-3′ and 5′-d$(T)_{10}C(T)_{10}$-3′. Each solution contained 1 μM of the oligomer in 10 mM Mops, 100 mM $NaNO_3$, pH 7.1. --- in the absence of metal, -□- $AgNO_3$ (2 μM), -■- $Hg(ClO_4)_2$ (2 μM), -△- $CuCl_2$ (2 μM). T_m of 5′-$(dA)_{21}$-3′/5′-T_{21}-3′ duplex at 44 °C and was unaffected by the addition of metal ions. Right: predicted structures of the metallo-base pairs.

the 1-deazaadenine-Ag(I)-T pair[75] and the scheme for F-Ag(I)-C (F = 5-fluorouracil) will be discussed in Section 3.2. Similarly, the imino proton of the thymine residue replaced by a Hg(II) ion, combined with the cytosine, coordinates the Hg(II) to form a T-Hg(II)-C pair. In the metallo-base pair, acidity of the proton of the cytosine exocyclic amino group could be increased by Hg(II) coordination.[24,78] In addition, if the proton is dissociated the metallo-base pair becomes neutral. Recently, DFT calculations by Megger and collaborators[79] suggested that the deprotonation of an exocyclic amino group can be disfavored even if this would reduce the charge on the base pair, thymine-$Ag(I)_2$-dideazaadenine. Structures of metallo-base pairs containing cytosine bases, C-Ag(I)-C and T-Ag(I)-C, have yet to be elucidated.

3.2 5-Fluorouracil–5-fluorouracil pair and the 5-fluorouracil–cytosine pair

Besides for its bioactivity as an anticancer drug, we have been interested in the chemical behavior of 5-fluorouracil in DNA strands. DNA strands containing 5-fluorouracil residues can be readily synthesized using the general protocol for preparing unmodified DNA strands with a commercially available 5-fluoro-2′-deoxyuridine-3′-phosphoramidite.

In acidic and neutral solutions, a Hg(II) ion binds to a fluorouracil–fluorouracil (F–F) pair in a duplex and thermally stabilizes duplex formation.[80] In neutral solution, the duplex was stabilized moderately in the presence of Ag(I) ions (Fig. 13A). In basic solution, the duplex containing the F–F pair was highly stable in the presence of Ag(I) ions (Fig. 13B). The duplex was fully stabilized in the presence of two equivalents of Ag(I) ions in the basic solution.[80] As mentioned in Section 3.1, a duplex containing a T–C pair was stabilized moderately in the presence of Ag(I) ions, forming a T-Ag(I)-C pair. The duplex containing a F–C pair was largely stabilized in the presence of Ag(I) ions and the stabilization effect of Ag(I) ions increased as the solution pH changed from pH 7 to pH 9 (Fig. 13C and D).

Proposed binding schemes for the metal ions and F–F pairs in duplexes are shown schematically in Fig. 14. The difference in pK_a values between thymine and 5-fluorouracil should be considered when designing binding schemes. Because of the electro-withdrawing property of the 5-fluoro substituting group, the electron-density of the uracil ring is decreased and the acidity of the imino proton is increased.[24] In acidic and neutral solutions, a Hg(II) ion, but not a Ag(I) ion, binds tightly to a F–F pair. In these solutions, 5-fluorouracil residues are neutral, and the imino protons are not dissociated, as in thymine residues. A binding mechanism similar to the

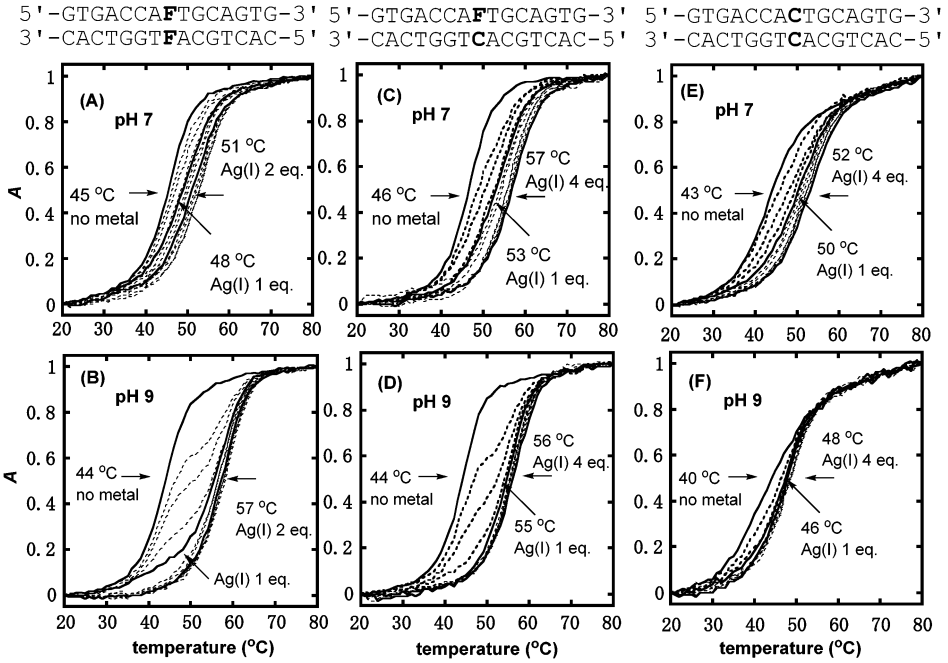

Fig. 13 Thermal denaturation profiles of the duplexes in the presence of various concentrations of Ag(I) ions, in 10 mM Mops and 100 mM $NaNO_3$.

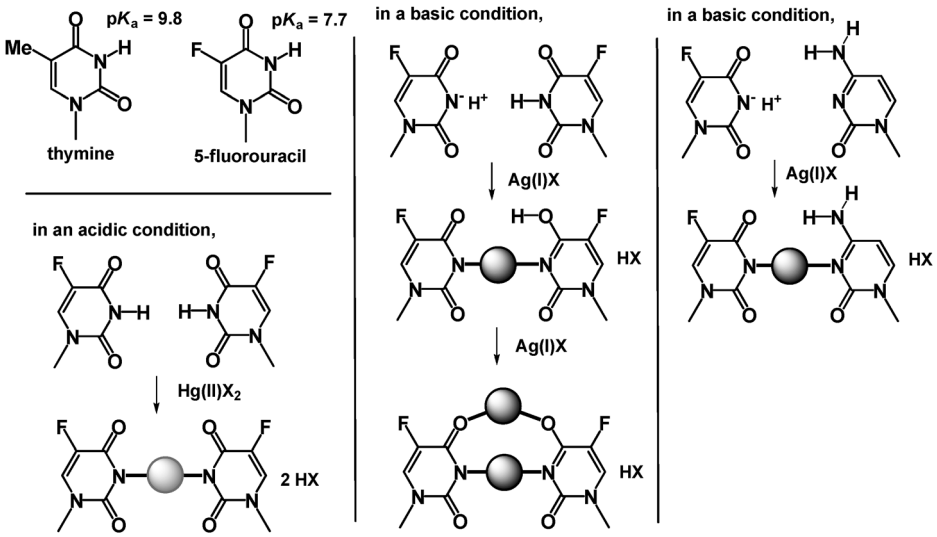

Fig. 14 Schematic representations of metallo-base pair formations involving 5-fluorouracil.

T-Hg(II)-T pair can be applied to the F-Hg(II)-F pair (Fig. 14). On the other hand, due to its smaller pK_a value, the imino protons of 5-fluorouracil residues are partially dissociated in basic solutions. A silver ion binds to the N3 position in the anion form; the N3 of the opposite base then coordinates the silver ion. Thus, the silver ion is captured between the uracil rings. The enol proton, which becomes more acidic with N3–Ag binding, dissociates to produce an enolate, and a second silver ion binds between the enolate and the 4-carbonyl group of the opposite residue (Fig. 14). Binding schemes in which two Ag(I) ions are placed between pyrimidine bases have been suggested theoretically[81] and have been experimentally demonstrated for Ag(I) ion binding to the thymine–dideazaadenine pair[79] and thiopyrimidine–thiopyrimidine pairs (Section 3.3).

In F-Ag(I)-C pair formation, one silver ion binds to the N3 position in the anion form, and the N3 in the cytosine residue coordinates the silver ion. Thus, the silver ion is captured between the 5-fluorouracil and cytosine residues (Fig. 14).

At pH 9, in the presence of less than one equivalent of a Ag(I) ion, two characteristic phase transitions were observed in the denaturation profiles of the duplex containing the F–C pair (Fig. 13D). Similar two-phased transition curves have been reported in the thermal denaturation profiles of duplexes having artificial base pairs, which tightly capture metal ions.[82] A silver ion binds tightly to the F–C pair and stays between the pyrimidine bases during the denaturation process. The free duplexes dissociate before the duplexes containing F-Ag(I)-C, which dissociate at a later time. Two phase transition curves observed in the denaturation profiles of the duplex containing the F–F pair (Fig. 13B) have been described in the literature.[80]

3.3 2-Thiothymine and 4-thiothymine

Metal ion binding of thiopyrimidine bases, such as 2-thiothymine and 4-thiothymine (Fig. 15), is worthy of investigation. In terms of the "hard and soft acids and bases" (HSAB) rule, it is anticipated that base pairs having sulfur atom(s) may be

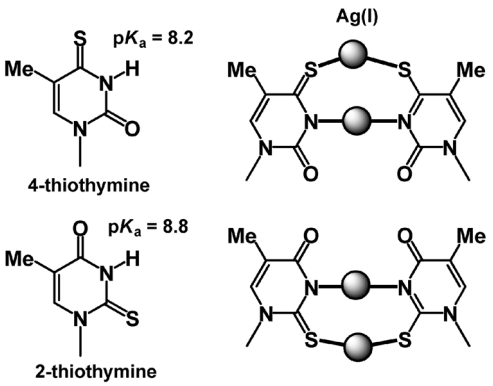

Fig. 15 Structures and expected metal ion binding schemes of the thiothymidines.

able to incorporate various heavy metal ions. However, few studies have reported metal ion binding by nucleosides containing sulfur atoms. Zimmermann and collaborators[83] reported silver ion binding to an artificial base pair having methylthio-side chains in a duplex. Takezawa and collaborators[84] reported that nucleosides having mercaptopyridione or hydroxypyridinethione captured Pd(II) and Ni(II) ions, and they expected that artificial nucleosides, once incorporated into DNA strands, may capture metal ions efficiently in a programmable fashion. In thermal denaturation experiments, duplexes containing a 2-thiothymine–2-thiothymine (2S–2S) pair or a 4-thiothymine–4-thiothymine (4S–4S) pair were stabilized in the presence of both Hg(II) ions and Ag(I) ions, demonstrating that both metal ions bound tightly to the base pairs.[85] Interestingly, the duplexes were fully stabilized in the presence of two equivalents of Ag(I) ions, even in a neutral solution. Predicted binding schemes are shown in Fig. 15. The pK_a values of the thiothymines are smaller than that of thymine. The thiocarbonyl group, a soft donor atom, tends to bind to a soft metal ion. Both effects may accelerate and stabilize 2S-Ag(I)$_2$-2S and 4S-Ag(I)$_2$-4S pairs. Signals corresponding to 2 : 1 complexes of Ag(I) ions and duplexes were detected in ESI-MS measurements.[86]

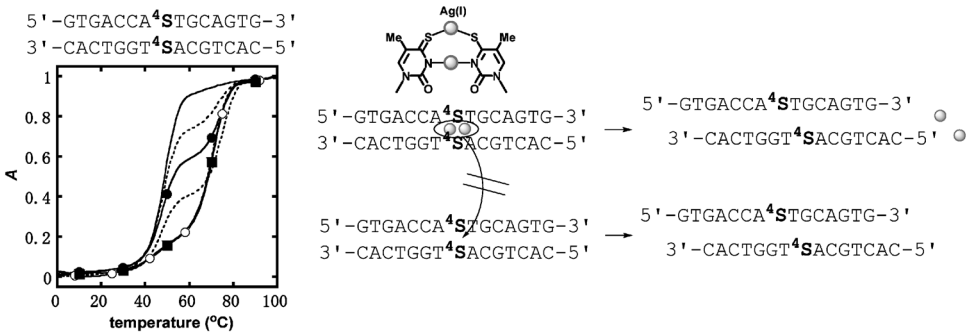

Fig. 16 Left: thermal denaturation profiles of the duplex (2 μM) in the presence of various amounts of Ag(I) ions: no metal, solid line, 1 μM Ag(I) -●-, 2 μM Ag(I) -○-, 3 μM Ag(I) -■-, in 10 mM Mops, pH 7, 100 mM $NaNO_3$. Right: Schematic representation of the denaturation process of a duplex containing a 4S-Ag(I)$_2$-4S pair.

As an example, thermal denaturation profiles of a duplex containing the 4S–4S pair in the presence of various amounts of Ag(I) ions are shown in Fig. 16. In the denaturation profile in the presence of one equivalent of a Ag(I) ion (Fig. 16, -●-), two transitions are observed. The transition curve at the higher temperature may correspond to the dissociation of a duplex containing 4S-Ag(I)$_2$-4S (having two silver ions) while the lower-temperature transition may correspond to the dissociation of a metal-free duplex. However, a transition curve corresponding to a duplex containing 4S-Ag(I)-4S (having one silver ion) was not observed. This phenomenon, combined with the ESI-MS experiment in which peaks for duplexes containing 4S-Ag(I)$_2$-4S were observed, indicates that two Ag(I) ions bind a 4S–4S pair to form a stable 4S-Ag(I)$_2$-4S pair. Very recently, it was reported that the distance between two Ag(I) ions in the metallo-base pair, dideazaadenine-Ag(I)$_2$-thymine, is shorter than the sum of their van der Waals radii. Thus "argentophilic d^{10}–d^{10} interactions" could occur.[79] Structures and properties of metallo-base pairs containing plural metal ions may be worth investigating.

4. Conclusions

The phenomenon that the naturally occurring pyrimidine base pairs, such as T–T and C–C, can selectively capture heavy metal ions has been applied to various scientific fields, ranging from basic theoretical studies to the applications discussed above. Metal ion binding motifs can be prepared from commercially available synthetic DNA fragments and can be readily used in many laboratories. Methods for detecting metal ion–duplex interactions, structures, and thermodynamic properties of metallo-base pairs were outlined. We also suggest that the strand orientation in specific duplexes containing (or consisting of) metallo-base pair(s) can be parallel, and thus the strand orientation (anti-parallel or parallel) should be carefully examined because structures and metal ion arrangements differ in the different orientations. With slight modifications to the pyrimidine bases, the metal ion binding properties of pyrimidine pairs can be significantly altered. Oligonucleotides containing the pyrimidine bases featured in this manuscript are commercially available and are therefore able to be used in many laboratories. We hope that this information will be useful for laboratories in various scientific fields.

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