Metal–DNA Complexes

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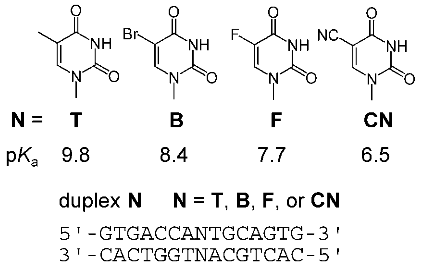
Metal-Ion Selectivity of Chemically Modified Uracil Pairs in DNA Duplexes**

*Itaru Okamoto, Kenji Iwamoto, Yuko Watanabe, Yoko Miyake, and Akira Ono**

There has been a great deal of research effort toward extending the functionality of base pairs in DNA duplexes with respect to artificial gene control and the development of DNA-based functionalized biopolymers.[1] Recently, synthetic oligodeoxyribonucleotides (ODNs) containing artificial bases have been used for forming metal-mediated base pairs in which hydrogen bonds in Watson–Crick (W–C) type base pairs in natural DNA are replaced by metal–base bonds.[1] Alternative methods for generating metal-mediated base pairs in DNA duplexes using only naturally occurring pyrimidine bases have also been reported.[2] That is, thymine–thymine (**T**–**T**) and cytosine–cytosine (**C**–**C**) base pairs selectively capture mercury(II)[2a–c,f] and silver(I) ions,[2d,e] and the metal-mediated base pairs **T**-Hg-**T** and **C**-Ag-**C**, respectively, are formed in DNA duplexes. As mercury–(**T**–**T**) pair binding is highly selective, novel DNA-based sensors capable of selectively detecting Hg^{II} ions in aqueous solutions containing various heavy metal ions have been developed.[3] Consequently, there is a great deal of interest in methods for capturing desired metal ions in DNA strands, as these are variable for developing new materials, such as DNA base wires containing metal ions[1] and new sensors capable of detecting various metal ions in aqueous solution.[2d,3] To develop methods for binding metal ions, various modified bases have been developed and introduced into DNA strands. Herein, we report a new method for changing metal-ion selectivity by varying the acidity of the uracil base without altering its skeleton.

We have reported a method for changing the acidity of thymine residues in DNA duplexes by chemical modification without significantly altering the DNA duplex structure.[4] When we substituted halogen and cyano groups for the 5-methyl group of the thymidine residue, the pK_a of the imino proton varied from 9.8 to 6.5, depending on the electron-withdrawing effects of the corresponding functional group (Scheme 1). As metal ions such as Hg^{II} and Ag^{I} bind between the N^3 atoms of pyrimidine bases,[2] the substituting groups may not come into direct contact with the metal ions. As the substituents at the 5-position of pyrimidine residues are placed in the major groove of the DNA duplex structure and face the outside of the helix, the substituents do not alter the duplex structure.[4,5]



Scheme 1. Structures of 5-substituted uracils (**N**) and DNA duplex containing **N**.

The thermal stabilities of duplex **N** in the presence of metal ions in solution at various pH values were determined by thermal denaturation experiments. Sharp transition curves were observed in all experiments (see the Supporting Information). Selected transition temperatures T_m are shown in Table 1. At all pH values, the transition curves of duplex **T**, which contained one **T**–**T** pair, were shifted to higher temperature ranges in the presence of Hg^{II}, and thus duplex **T** was stabilized in the presence of Hg^{II} ions over the whole range of pH values examined in this experiment, consistent with previous observations.[2a] In contrast, addition of Ag^{I} ions did not markedly stabilize duplex **T**, except for a slight stabilization at pH 9. Other duplexes, however, were markedly stabilized in the presence of Ag^{I} ions in neutral and basic solutions.

Figure 1 shows T_m values of the duplexes versus pH value in the presence and absence of the metal ions. In the absence of metal ions, all duplexes were most stable at neutral pH values and became unstable in both acidic and basic solutions, as Watson–Crick base pairs were destabilized in both acidic and basic solutions (Figure 1 A–D). Over the whole range of pH values examined herein, duplex **T** was stabilized in the presence of Hg^{II} (Figure 1 A). In contrast, Ag^{I} did not have any obvious effects on T_m values, except that they increased slightly in the presence of Ag^{I} in basic solution. The

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[*] Dr. I. Okamoto, K. Iwamoto, Dr. A. Ono
Department of Material and Life Chemistry
Faculty of Engineering, Kanagawa University
3-27-1 Rokkakubashi, Kanagawa-ku
Yokohama, Kanagawa 221-8686 (Japan)
Fax: (+81) 45-481-7915
E-mail: akiraono@kanagawa-u.ac.jp
Homepage:
http://apchem2.kanagawa-u.ac.jp/~onolab/welcome.html
Y. Watanabe, Dr. Y. Miyake
Department of Chemistry, Graduate School of Science
Tokyo Metropolitan University
Minamiohsawa 1-1, Hachioji, Tokyo 192-0397 (Japan)

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Table 1: T_m values of the thermal transitions of duplex **N** in the presence and absence of metal ions in solutions at various pH values.[a]

Duplex	Additives	T_m [°C] pH 5.5	 pH 7.1	 pH 9.0
	–	47	49	48
duplex **T**	Hg^{II}	53	57	54
	Ag^{I}	48	50	50
	–	47	48	41
duplex **B**	Hg^{II}	55	56	51
	Ag^{I}	48	53	55
	–	44	47	41
duplex **F**	Hg^{II}	51	54	47
	Ag^{I}	45	52	55
	–	42	44	39
duplex **CN**	Hg^{II}	48	50	40
	Ag^{I}	45	57	53

[a] Each solution contained 2 μM duplex **N**, 4 μM metal ion, 10 mM appropriate buffer, and 100 mM $NaNO_3$. Sodium cacodylate HCl buffer was used for pH 5.5 and 7.0, and boric acid NaOH buffer was used for pH 9.0.

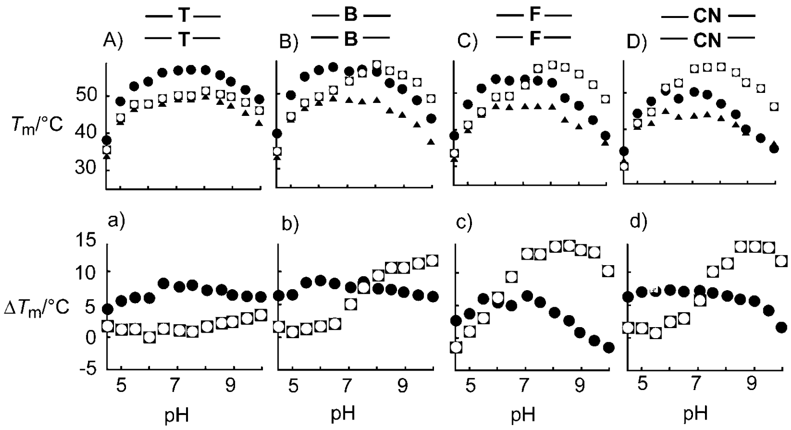


Figure 1. A–D) T_m vs. pH value. a–d) ΔT_m vs. pH value. A, a) duplex **T**; B, b) duplex **B**; C, c) duplex **F**; D, d) duplex **CN**. ▲ No metal ions; ● Hg^{II} ions; □ Ag^{I} ions.

profiles of T_m versus pH for duplex **B** containing the 5-bromouracil pair (**B**–**B**) instead of the **T**–**T** pair were similar to those of duplex **T** in the absence of metal ions or in the presence of Hg^{II} (Figure 1B). However, as the pH value increased above pH 7, the T_m values in the presence of Ag^{I} increased in a stepwise manner and reached the highest value at around pH 8.5. The T_m value began to decrease in solutions with pH value higher than 9, as W–C-type base pairs are destabilized in basic solution. Even in such basic solution, the ΔT_m value [$\Delta T_m = (T_m$ in the presence of the metal ion$) - (T_m$ in the absence of the metal ion$)$] was maintained (Figure 1b), which may indicate that the **B**-Ag-**B** pair is stable in basic solutions in which W–C-type base pairs are destabilized.

Similar phenomena were observed for duplex **F** containing the 5-fluorouracil pair (**F**–**F**), the pK_a of which is lower than those of **B** and **T**. In the presence of Ag^{I}, T_m values began to increase at pH 7 and reached the highest values at around pH 8 (Figure 1C), and ΔT_m values were maintained in the higher pH range (Figure 1c). The duplex containing **CN**, which is the most acidic of the 5-substituted uracils, showed the most characteristic properties (Figure 1D, d). In the presence of Ag^{I}, T_m began increase at pH 6 and reached the highest value at pH 7–8, and ΔT_m was maintained at higher pH values. Addition of Hg^{II} ions did not markedly stabilize duplex **CN** over the whole pH range, especially in basic solution. Consequently, all duplex **N** species were stabilized in the presence of Ag^{I} ions at pH values close to the pK_a values of the **N** residues.

The thermal denaturation profiles of duplex **F** in the presence of various concentrations of Ag^{I} ions are shown in Figure 2. At pH 7, the thermally induced transitions shifted to higher temperature as the concentration of Ag^{I} ions increased (Figure 2A). The plot of T_m versus Ag^{I} ion concentration suggested that two Ag^{I} ions bind to the **F**–**F** pair, fully stabilizing duplex formation (Figure 2a). At pH 9.0, two transitions were observed in dissociation profiles for which less than two equivalents Ag^{I} were present relative to the **F**–**F** pair (Figure 2B), and thus proper T_m values could not determined (Figure 2b).[6] However, it was suggested that duplex **F** was fully stabilized with two equivalents Ag^{I} ions. Consequently, it was supposed that the (**F**–**F**)–$(Ag)_2$ complex was much more stable than the (**F**–**F**)–Ag complex, at least in basic solution.

Scheme 2 shows the proposed binding scheme for the **N**–**N** pairs and the metal ions. In the crystal structure of a 2:1 complex of 1-methylthymine Hg^{II}, a covalent and linear N^3-Hg-N^3 bond was observed.[7] The results of ^1H and ^{15}N NMR spectroscopy experiments supported the suggestion that the binding scheme was reproduced in formation of the **T**-Hg^{II}-**T** complex in DNA duplexes.[2a,c,f] Consequently, the **N**-Hg-**N** pairs may be neutral, and a covalent and linear N^3-Hg-N^3 bond may be the main contact in the base pair (Scheme 2a). The determined ΔT_m values were similar over the whole range of examined pH values (Figure 1a–c), except that Hg^{II} did not stabilize duplex **CN** at pH > 9 (Figure 1d). The **CN**-Hg-**CN** pair was less stable than the other **N**-Hg-**N** pairs over the whole range of pH values, probably because the low electron density of the 5-cyanouracil ring weakens the **CN**−Hg bond. Moreover, the **F**-Hg-**F** pair was destabilized at pH > 9 because the **F**−Hg bond was weaker than the **B**−Hg and **T**−Hg bonds, and the electron density of **F** is lower than those of **B** and **T** owing to the strong electron-withdrawing effect of the 5-fluoro group.

In the **C**–**C** pair, the Ag^{I} ion is expected to bind between the N^3 imino groups, and the resulting **C**-Ag^{I}-**C** complex will still carry a positive charge (Scheme 2b).[2d] The observation that each duplex **N** was not stabilized in the presence of Ag^{I} at pH values below the pK_a value of each species **N** suggests that Ag^{I} does not bind to the neutral imino form of **N**. However, at pH values around and higher than the pK_a value of each **N**, each duplex **N** was stabilized in the presence of Ag^{I} ions. Therefore, it was assumed that one Ag^{I} ion binds to the N^3

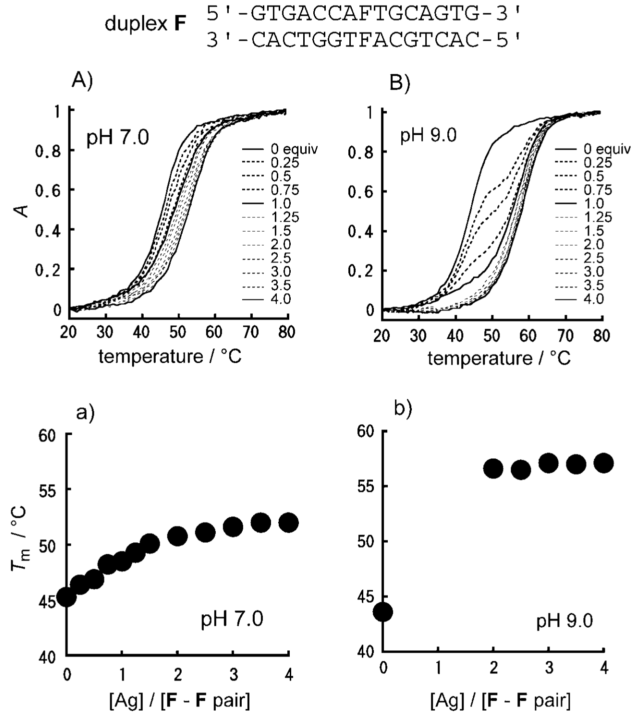
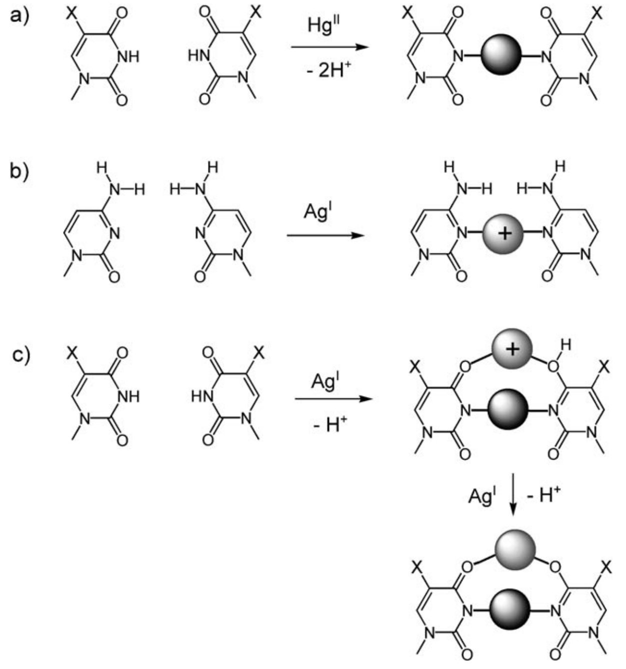


Figure 2. A, B) Thermally induced transition profiles of duplex **F** in solutions containing various concentrations of Ag^{I} ions (0 to 4.0 equivalents) at pH 7 (A) and pH 9 (B). a, b) T_m vs. Ag^{I} concentrations at pH 7 (a) and pH 9 (b).



Scheme 2. Proposed binding schemes.

position of one pyrimidine base (replacing a proton with silver), and the N^3 atom of the opposite pyrimidine base coordinates to the Ag^{I} ion, forming a N^3-Ag^{I}-N^3 bridge. Another Ag^{I} ion may bind between the keto and enol groups (Scheme 2c). In basic solutions, deprotonation from the enol group neutralizes the **N**-(Ag)$_2$-**N** complex, and so duplex **N** was still stable in the presence of Ag^{I} ions, even in basic solutions in which the W–C base pairs were destabilized. Efforts are underway to determine the exact coordination geometry of the new base pairs by X-ray crystallographic analysis.

Herein, we proposed a new strategy for selectively controlling metal–base-pair bonding by changing the acidity of the 5-substituted uracil units by precise chemical modifications. The strategies reported herein will be useful for the development of new molecular architectures with novel functions.[1,8] For example, methods for programmable assembly of metal ions along DNA duplex structures, which were established using the artificial bases as metal-ion binding sites,[1f] could be developed using only natural pyrimidine bases based on the phenomenon that **T**–**T** and **C**–**C** pairs highly selectively bind Hg^{II} and Ag^{I} ions, respectively.

Furthermore, using the 5-modified uracil pairs, the assembled metal ions can be exchanged by changing the pH value, as demonstrated in Figure 3. In a solution containing both Hg^{II} and Ag^{I} ions at pH 7, the Hg^{I} ion preferentially binds to duplex **F**, as confirmed by ESI-MS. By contrast, at pH 9, the Ag^{I} ion preferentially binds to duplex **F**. A study to develop a method to arrange metal ions in a given order that can be changed in response to an external stimulus, such as a change in pH value, is in progress.

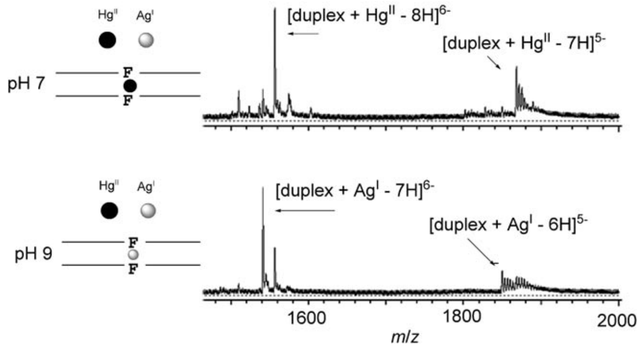


Figure 3. ESI mass spectra of duplex **F** in the presence of Hg^{II} and Ag^{I} ions at pH 7 and pH 9.

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