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Metal-Mediated DNA Base Pairing: Alternatives to Hydrogen-Bonded Watson–Crick Base Pairs

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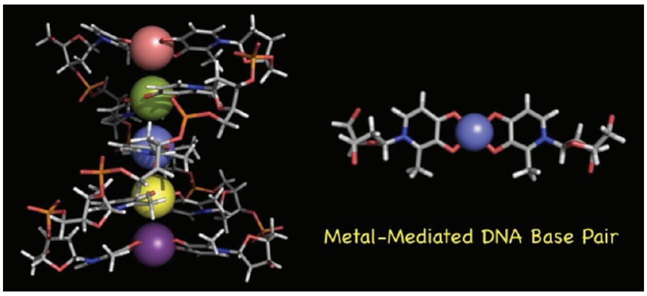

With its capacity to store and transfer the genetic information within a sequence of monomers, DNA forms its central role in chemical evolution through replication and amplification. This elegant behavior is largely based on highly specific molecular recognition between nucleobases through the specific hydrogen bonds in the Watson–Crick base pairing system. While the native base pairs have been amazingly sophisticated through the long history of evolution, synthetic chemists have devoted considerable efforts to create alternative base pairing systems in recent decades. Most of these new systems were designed based on the shape complementarity of the pairs or the rearrangement of hydrogen-bonding patterns. We wondered whether metal coordination could serve as an alternative driving force for DNA base pairing and why hydrogen bonding was selected on Earth in the course of molecular evolution. Therefore, we envisioned an alternative design strategy: we replaced hydrogen bonding with another important scheme in biological systems, metal-coordination bonding.

In this Account, we provide an overview of the chemistry of metal-mediated base pairing including basic concepts, molecular design, characteristic structures and properties, and possible applications of DNA-based molecular systems. We describe several examples of artificial metal-mediated base pairs, such as Cu^{2+}-mediated hydroxypyridone base pair, $H-Cu^{2+}-H$ (where H denotes a hydroxypyridone-bearing nucleoside), developed by us and other researchers. To design the metallo-base pairs we carefully chose appropriate combinations of ligand-bearing nucleosides and metal ions. As expected from their stronger bonding through metal coordination, DNA duplexes possessing metallo-base pairs exhibited higher thermal stability than natural hydrogen-bonded DNAs. Furthermore, we could also use metal-mediated base pairs to construct or induce other high-order structures. These features could lead to metal-responsive functional DNA molecules such as artificial DNAzymes and DNA machines. In addition, the metallo-base pairing system is a powerful tool for the construction of homogeneous and heterogeneous metal arrays, which can lead to DNA-based nanomaterials such as electronic wires and magnetic devices. Recently researchers have investigated these systems as enzyme replacements, which may offer an additional contribution to chemical biology and synthetic biology through the expansion of the genetic alphabet.

Introduction

A wide range of biomolecular structures, which are the products of longtime molecular evolution, are mostly formed based on supramolecular association with the aid of noncovalent interactions such as hydrogen bonding, hydrophobic effect, and metal-coordination bonding. As we can already see, hydrogen bonding contributes greatly to self-folding and multiassembly of biomolecules through their highly specific intra- and intermolecular recognition and reversible, dynamic bonding. Metal coordination also plays various roles in maintaining and stabilizing biologically important structures in terms of molecular recognition and catalytic activity. Such noncovalent interactions often provide a great option to synthetic chemistry, which may possibly lead to a dramatic evolution of artificial supermolecules with "intelligent" functions that had been found only in biological systems.[1] Metal-coordination bonding has been extensively used for the construction of many types of biomolecule-like supramolecular architectures such as single-stranded peptide-like foldamers, double-stranded DNA-like helicates,[2,3] and even virus-like molecular capsules,[4] taking advantage of its structural diversity and kinetic reversibility. Especially when one thinks

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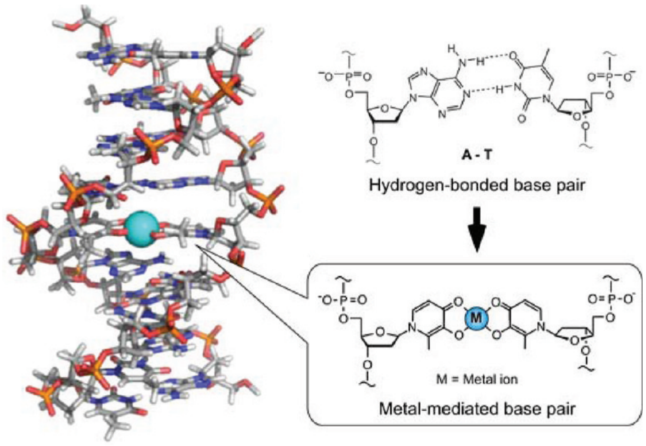

FIGURE 1. Artificial metal-mediated base pairing system (a computer-generated model).

of prebiotic molecular evolution interfacing inorganic materials and organic biomolecules, to study the fundamental processes of metal-mediated self-assembly and subsequent expression of molecular functions can be referred to as an essential synthetic approach to the "origins of chemical evolution".

There is no doubt that one of the most fundamental biomolecules involved in the origins of chemical evolution is the nucleic acid, which stores, transfers, and replicates the genetic information based on the hydrogen-bonded Watson–Crick base pairing system. Consequently, it is a fascinating challenge to address a question whether hydrogen bonding between natural DNA base pairs can be replaced by metal-coordination bonding. This approach provides valuable insights into the origin of chemical evolution that might involve both inorganic and organic compounds, and it leads to completely different, alternative base pairs consisting of both inorganic and organic species, namely, metal-mediated base pairs (Figure 1).[5,6] Compared with other types of unnatural base pairs based on rearrangement of the hydrogen-bonding pattern or shape complementarity,[7] metal-mediated base pairs have an advantage in that many types of metal ions can be incorporated in light of their unique chemical and physical properties. Accordingly, well-thought-out selection of metal species would enable us to adjust appropriately the coordination properties of the metallo-base pairs, namely their selectivity and structures as well as thermodynamic stability.

In this Account, we give an overview of the development of metal-mediated base pairing systems to date, including their concept, design strategy, and characteristic structures and properties as alternatives for hydrogen-bonded base pairs, and discuss possible applications and contributions to evolve intelligent molecules that might supersede native biomolecules.

Design of Metal-Mediated Base Pairing Systems

The simplest idea leading to the design of metal-mediated base pairs was to replace the hydrogen atom within hydrogen-bonded base pairs by a metal ion, based on the fact that both accept lone pairs on the heteroatoms. In 1993, Lee et al. found that, at elevated pH, some divalent metal ions such as Zn^{2+} ions stabilize DNA duplexes,[8] in which hydrogen atoms involved in the base pairs were released and the metal ions were bound to the nucleobases. The next idea to utilize metal ions for base pairing is the conversion of a natural mismatched base pair into a metal-coordinated matching pair. For example, it is well-known that a T–T mismatch pair is altered to a stable, neutral metallo-base pair in the presence of Hg^{2+} ions, as originally proposed by Karz et al.[9] and further studied by Marzilli et al.[10] Recently, Ono et al. shed light on the T–Hg^{2+}–T pair (**1**) again and proceeded with further investigation.[11] In a similar way, a C–C mismatch pair can be transformed into a metallo-base pair (C–Ag^{+}–C, **2**).[12] Such metal-mediated base pairing is now widely applied for metal-responsive functional DNA molecules including allosteric DNAzymes[13] and logic gates.[14] Although the very idea of replacing the hydrogens involved in natural base pairs with metals is quite simple, this would make the complementarity rule of the base pairing inconveniently jumbled up. In other words, such metal-mediated base pairs with natural nucleobases could not become orthogonal to natural base pairs. This drawback provided us a strong incentive to develop a novel metal-mediated base-paring system using completely synthetic ligand-type nucleobases.

Our artificial metallo-base pairs are designed to meet the structural requirement that their size and shape fit to those of canonical base pairs, as is the case with hydrogen-bonded or hydrophobic unnatural base pairs. Most importantly for the metallo-base pairing, the centrally positioned metal ion should be chosen deliberately so that the resulting base pairs prefer planar coordination geometries and can be stacked on top of each other without greatly perturbing the DNA duplex structure. For example, linear-coordinating Ag^{+} and Hg^{2+} ions and square-planar-coordinating Cu^{2+}, Ni^{2+}, Pd^{2+}, and Pt^{2+} ions were the first candidates from a series of transition metals. It should be also noted that the net charge of the metal complexes should be considered in designing metallo-base pairs that ought to be incorporated into the hydrophobic interior of DNA duplexes.

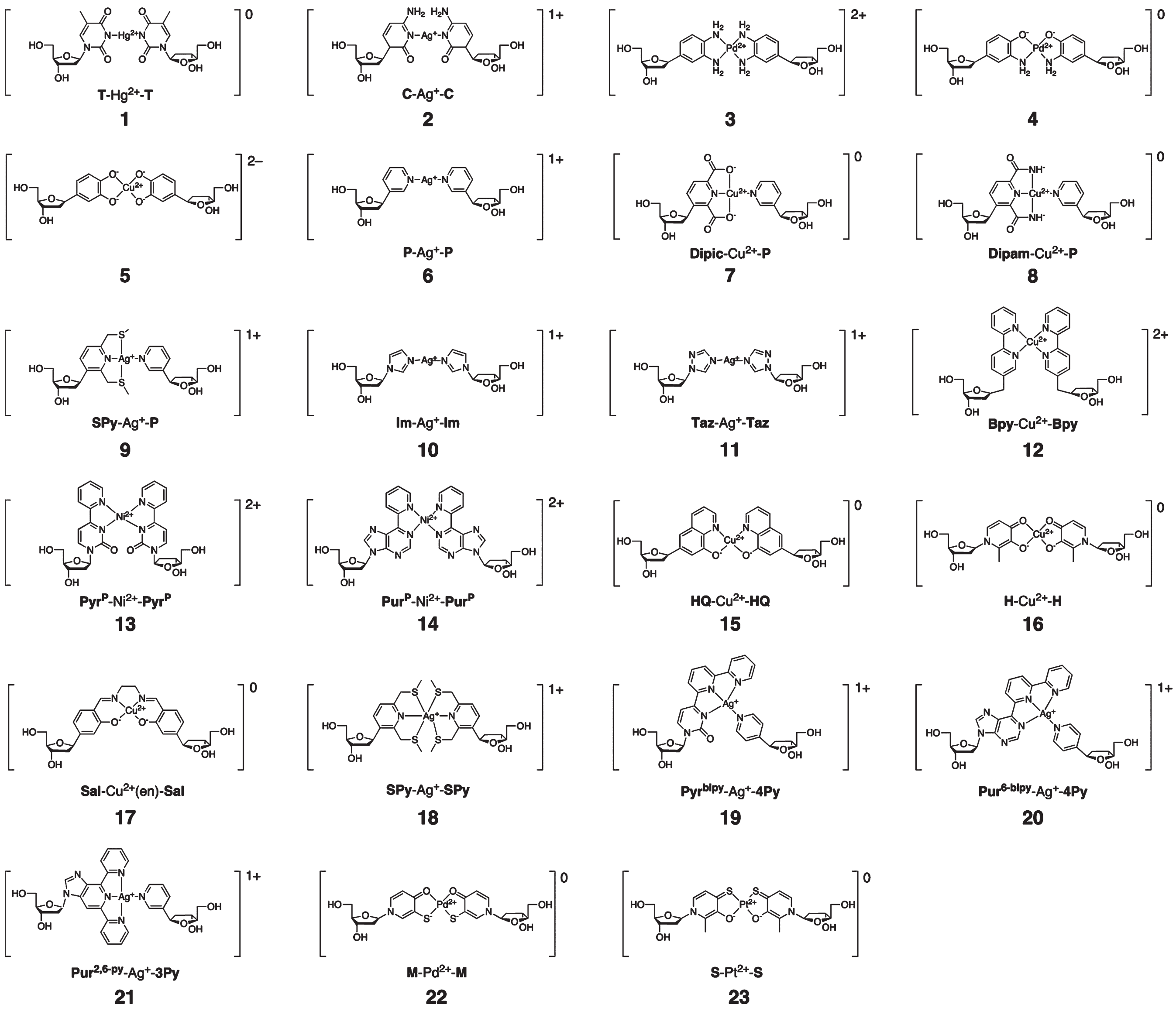

FIGURE 2. Alternative metal-mediated base pairs. Detailed coordination structures are not experimentally supported for some base pairs.

Since we developed the first metallo-base pair (**3**) in 1999,[15] a variety of excellent examples have been reported (Figure 2).[5,16−20] Most of them are efficiently incorporated into DNA duplexes by the standard phosphoramidite chemistry using automated DNA synthesizers, and their properties were investigated as will hereinafter be described. One of the most successful metallo-base pairs so far is formed by hydroxypyridone-bearing nucleosides (**H**) and a Cu^{2+} ion, reported by our group in 2002.[21,22] The base pair **H**−Cu^{2+}−**H** (**16**) has a flat, square-planar structure and has no charge due to the deprotonation of the α-hydroxyl groups upon metal coordination, so it is considered to fit well with hydrophobic base stacked areas within DNA duplex. In addition, it is noteworthy that the complex is sufficiently stable at a concentration ranging from 10^{-5} to 10^{-6} M at neutral pH, which is a common condition for biochemical experiments and applications.

The versatility of metal-mediated base pairing is additionally supported by the fact that it becomes broadly adopted even for DNA analogues such as peptide nucleic acids (PNA)[23] and glycol nucleic acids (GNA) comprised of acyclic propylene glycol phosphodiester backbones.[24] For example, Meggers et al. incorporated **H**−Cu^{2+}−**H** and other metallo-base pairs into GNA double strands to study their structures and stabilities.

Stabilization of DNA Duplexes by Metal-Mediated Base Pairs

The most distinctive feature of the metallo-base pairs incorporated inside DNA is a potential to increase overall duplex stability, as is expected from the fact that bond energies of metal coordination are generally twice or three times larger than those of hydrogen bonds. Their stabilization effect can be estimated by melting temperatures (T_m) of the DNA duplexes, defined as a midpoint of thermal transition from duplex to single strand, in a manner similar to the other DNA conjugates. The T_m value of a 15-mer DNA duplex containing hydroxypyridone nucleotides (**H**), d(5′-CACATTA**H**TGTTGTA-3′)·d(3′-GTGTAAT**H**ACAACAT-5′), was found to be 37.0 °C without Cu^{2+} ions, indicating that the **H**–**H** base pair behaved as a mismatch pair and destabilized the duplex.[21] In the presence of 1 equiv of Cu^{2+} ions (i.e., $[Cu^{2+}]/[\text{DNA duplex}] = 1.0$), the T_m value was increased to 50.1 °C owing to the formation of the Cu^{2+}-mediated base pair. The difference in the T_m value between the metal-mediated "matched" pair (**H**–Cu^{2+}–**H**) and the metal-free "mismatched" pair (**H**–**H**), hereafter denoted as $\Delta T_m^{\text{metal}}$, is +13.1 °C, which is larger than those observed between the native match and mismatch pairs. More importantly, the difference in the T_m relative to DNA duplex with all Watson–Crick base pairs ($T_m = 44.2$ °C), hereafter denoted as ΔT_m^{WC}, is calculated as high as +5.9 °C. This value corresponds primarily to the larger bond energy of metal coordination bond compared with that of hydrogen bond, despite non-negligible stacking interactions with the neighboring base pairs. We would like to emphasize herein that only an equal amount of Cu^{2+} ion (i.e., $[Cu^{2+}]/[\mathbf{H}\text{–}\mathbf{H}] = 1$) is needed for stable **H**–Cu^{2+}–**H** base pairing under these conditions (2.0 μM duplex, pH 7.0), suggesting that the **H**–Cu^{2+}–**H** pair would provide an alternative DNA base pair for biological approaches.

The largest stabilization ($\Delta T_m^{\text{WC}} = +32.3$ °C) to date was achieved by a salen-type base pair, **Sal**–Cu^{2+}(en)–**Sal** (**17**), in which both metal coordination and a reversible covalent cross-linking with ethylenediamine (en) mediate the pairing.[25] Even without ethylenediamine, a neutral base pair, **Sal**–Cu^{2+}(en)–**Sal**, was formed with a ΔT_m^{WC} value of +4.8 °C, comparable to that of the **H**–Cu^{2+}–**H** pair. Both stabilization effects are attributed to a square-planar metal coordination. Addition of both an excess of ethylenediamine and 1 equiv of Cu^{2+} ion under basic conditions (pH 9.0) drastically increased the duplex stability ($T_m = 82.4$ °C) via the formation of a **Sal**–Cu^{2+}(en)–**Sal** base pair. Its large stabilization can be ascribed to the chelating effect upon metal binding and to a cooperative effect exerted by the covalent cross-linking with ethylenediamine and the metal coordination.

The melting temperature measurements showed that the incorporation of only a single metallo-base pair exhibits a remarkable stabilization of DNA duplex, which would be utilized for minimization of functional DNAs including aptamers and ribozymes. The stability of the metallo-DNAs can be controlled by means of addition or removal of metal ions without any changes in conditions such as temperature, thereby allowing switchable molecular systems such as DNA sensors and DNA computers. It is also worth noting that only thermodynamic stability has been the major concern in the development of metal-mediated base pairs, whereas metal coordination bonding varies a great deal in the rates of association and dissociation depending on the combination of metals and ligands. Given that the time-sensitive activity modulation of functional nucleic acids is an important factor in biological systems, kinetic behavior as well as long-term stability of metallo-base pairs should be comprehensively made clear in the future.

Structures of DNAs Containing Metal-Mediated Base Pairs

One of the most fundamental questions to be raised about the metallo-base pairs concerns their structures when incorporated into the DNA duplexes, because the detailed structural information is helpful to studies on compatibility in biological systems including enzymatic reactions and to the design of novel metallo-base pairs. The coordination structures of the metallo-base pairs incorporated into the DNA duplexes were initially estimated by their monomeric structures that can be readily analyzed by crystallographic methods and NMR or electron paramagnetic resonance (EPR) spectroscopy. For example, the EPR spectra of the DNA duplexes containing a **Dipic**–Cu^{2+}–**P** (**7**)[26] or an **H**–Cu^{2+}–**H** (**16**)[21] base pair confirmed the square-planar-like coordination with a doublet ($S = 1/2$) spin state, which corresponds to d^9 Cu^{2+} ions. Circular dichroism (CD) spectroscopy has been widely used to elucidate the entire secondary structures of the metallo-DNAs, indicating that most metal-mediated base pairs hardly affect the right-handed B-DNA structure.

The first structural analysis of the DNA duplex containing metallo-base pairs was achieved by Schultz et al. in 2001 using a palindromic dodecamer duplex, d(5′-CGCG**Dipic**-AT**P**CGCG-3′)$_2$, containing two **Dipic**–Cu^{2+}–**P** base pairs (Figure 3A).[27] Interestingly, the overall conformation of the duplex was found to be that of Z-DNA, a left-handed duplex, which was considered to be due to its alternating purine–pyrimidine sequence and to be a result of an additional axial coordination of an oxygen donor of the neighboring nucleoside to the Cu^{2+} ion. Very recently, Carell et al. determined the crystal structure of a duplex containing

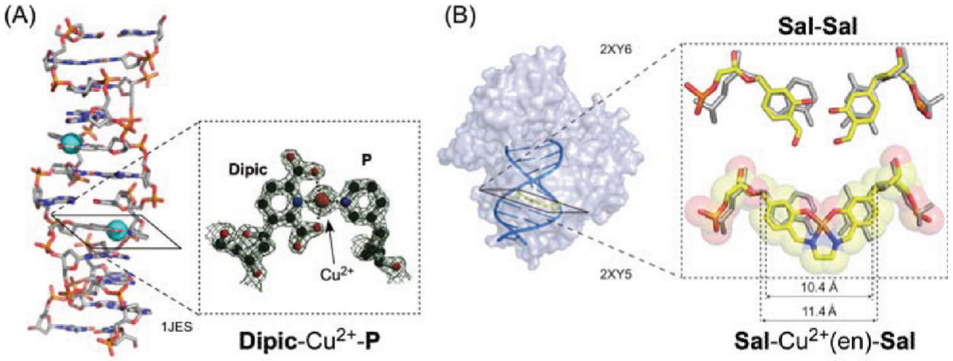

FIGURE 3. Crystal structures of DNA duplexes containing metal-mediated base pairs: (A) **Dipic**–Cu^{2+}–**P** base pairs in a palindromic dodecamer DNA duplex. Reproduced from ref 27 and drawn from PDB 1JES. (B) **Sal**–**Sal** mismatched base pairs and metal-mediated **Sal**–Cu^{2+}(en)–**Sal** base pairs inside a DNA polymerase, *Bst* Pol I (PDB 2XY5 and 2XY6). Native A–T base pairs are overlaid. Reproduced from ref 28. Copyright 2011 Nature Publishing Group.

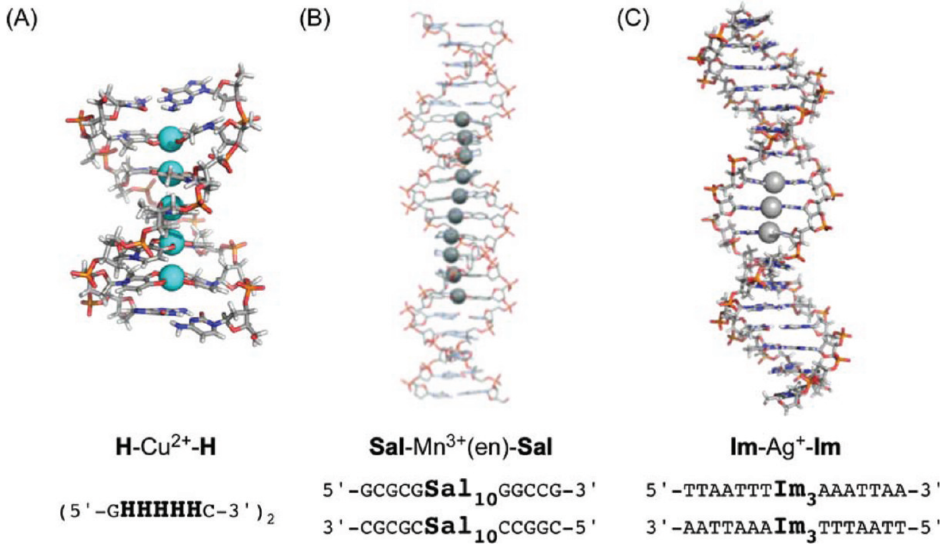

FIGURE 4. Multiple incorporation of consecutive metal-mediated base pairs: (A) Five **H**–Cu^{2+}–**H** base pairs (a computer-generated model). (B) Ten **Sal**–Mn^{3+}(en)–**Sal** base pairs (a computer-generated model). Reproduced from ref 30. Copyright 2007 Wiley-VCH. (C) Three **Im**–Ag^{+}–**Im** base pairs. Drawn from PDB 2KE8 (NMR structure).

salen-type base pair (**Sal**–Cu^{2+}(en)–**Sal**) inside a DNA polymerase, *Bst* Pol I (Figure 3B).[28] The **Sal**–Cu^{2+}(en)–**Sal** base pair was found to adopt a square-planar geometry as designed and to be sufficiently fit with the B-type duplex structure.

A solution structure of a DNA duplex with consecutive metallo-base pairs was disclosed by Müller and Sigel et al. by means of NMR spectroscopic methods including [^{1}H,^{15}N]-heteronuclear single quantum coherence (HSQC) and [^{1}H,^{1}H]-nuclear Overhauser enhancement spectroscopy (NOESY) analyses (Figure 4C).[29] Three **Im**–Ag^{+}–**Im** base pairs (**10**) were inserted into an A–T alternating palindromic sequences, d(5′-TTAATTT**ImImIm**AAATTAA-3′)$_2$, in which the imidazole nucleosides (**Im**) were labeled with ^{15}N isotopes. The observation of $^1J(^{15}\mathrm{N},^{107/109}\mathrm{Ag})$ couplings gave direct evidence of the formation of the **Im**–Ag^{+}–**Im** base pairs at the designed positions, and the NOE analysis revealed that the duplex adopts a right-handed B-DNA-like structure. The base pair parameters, such as helical twists (Ω) and helical rises (h), show a slight unwinding of the helix in the region of the artificial bases ($\Delta\Omega \approx -8°$ and $\Delta h =$ 0.7–0.9 Å).

As described above, there are only a few examples of structural characterization of metallo-DNAs, and discussions based on structural parameters remain insufficient. Thus, further experimental investigation, as well as molecular modeling, on their structure is one of the main issues.

Multiple Incorporation of Metal-Mediated Base Pairs into DNA Duplexes

From the viewpoint of synthetic biology, sequential incorporation of noncanonical base pairs including metallo-base pairs has been an intriguing research target because it allows the complete expansion of genetic codes, in which 125 (= 5^3) triplet codons are theoretically provided with a symmetrical artificial base pair (e.g., **H**–Cu^{2+}–**H**) or 216 (= 6^3) codons with an unsymmetrical pair (e.g., **Dipic**–Cu^{2+}–**P**). Especially for the metal-mediated base pairs, to answer the question of whether all interstrand hydrogen bonds in the DNA duplexes can be replaced by metal coordination bond was an original motivation when we initiated this research. Thus, constructing artificial DNA helices not through hydrogen bonding but with consecutive metal-mediated base pairing was our first goal. Apart from the decrease in their yields, oligonucleotides possessing consecutive synthetic ligand-bearing nucleotides can be readily prepared by utilizing automated DNA synthesizers, and thereby, in principle, artificial DNA duplexes containing multiple metallo-base pairs can be obtained (Figure 4).[22,30–34]

The stacking of metallo-base pairs was first achieved in 2003 with consecutive, up to five, **H**–Cu^{2+}–**H** base pairs (Figure 4A).[22] A series of oligonucleotides possessing one to five hydroxypyridone-bearing nucleosides, d(5′-G**H**$_n$C-3′) ($n = 1-5$), did not form duplexes in the absence of metals due to the lack of enough natural nucleobases. In contrast, in the presence of Cu^{2+} ions, these artificial DNA strands formed double helices quantitatively through **H**–Cu^{2+}–**H** base pairing. The base pair formation was confirmed by UV-based titration experiments, whose spectra clearly showed the gradual increase in the absorbance at 307 nm ascribable to the formation of **H**–Cu^{2+}–**H** complexes and demonstrated the stoichiometric complexation (i.e., $n\mathrm{Cu}^{2+}\cdot$d(5′-G**H**$_n$C-3′)$_2$). The CD spectra suggested that the metallo-base pairs are stacked within the right-handed duplex structure, and the EPR spectra revealed that Cu^{2+}–Cu^{2+} distance is 3.7 ± 0.1 Å, which is comparable to that between natural base pairs (3.3–3.4 Å for B-DNA).

The salen-type artificial base pairs were also adjoiningly incorporated into the DNA duplexes in a manner similar to the **H**–Cu^{2+}–**H** base pairs. Up to 10 **Sal**–Mn^{3+}(en)–**Sal** base pairs were found to form in a way that they are stacked on top of each other inside B-DNA-like double helices (Figure 4B).[30] The distance between two adjacent metal centers was estimated to be 3.7 ± 0.1 Å for the consecutive **Sal**–Cu^{2+}(en)–**Sal** base pairs by EPR spectroscopic measurement.[31] Another example to be noted in this section concerns the **Im**–Ag^{+}–**Im** base pairs (**10**),[29] as already mentioned above (Figure 4C). Three consecutive **Im**–Ag^{+}–**Im** base pairs did not disturb the right-handed duplex structure despite a slight unwinding of the helix in the region of **Im**–Ag^{+}–**Im** stack. In addition, the calculated Ag^{+}–Ag^{+} distances between neighboring **Im**–Ag^{+}–**Im** base pairs were 3.92 and 3.97 Å for the lowest-energy structure, which may reflect the unwound helical structure.

Toward Information Storage: Sequence Control of Heterogeneous Metal-Mediated Base Pairs

Artificial base pairing systems that are orthogonal to Watson–Crick base pairs have been developed to expand the genetic information by combination of both canonical and noncanonical base pairs. Meanwhile, replacement of all natural base pairs by artificial ones is considered to provide alternative genetic coding systems, which is also an attractive research goal. In this regard, incorporation of more than one type of metal-mediated base pairs into the DNA duplex backbones holds promise to allow a completely synthetic information storage system in which all interstrand hydrogen bonds are replaced by metal coordination bonds.

We succeeded in the incorporation of two different types of metallo-base pairs, Cu^{2+}-mediated and Hg^{2+}-mediated base pairs, by using oligonucleotides possessing two different ligand-bearing nucleosides such as hydroxypyridone-type (**H**) and pyridine-type nucleosides (**P**) (Figure 5).[35] In the presence of both Cu^{2+} and Hg^{2+} ions, an artificial pentanucleotide d(5′-G**HPH**C-3′) formed a double helix, $2\mathrm{Cu}^{2+}\cdot\mathrm{Hg}^{2+}\cdot$d(5′-G**HPH**C-3′)$_2$, through metal-mediated base pairing, **H**–Cu^{2+}–**H** (**16**) and **P**–Hg^{2+}–**P**, which was confirmed by UV- and CD-spectroscopic titration experiments and by mass spectrometric characterization. As a result, a three-base-pair sequence "(**H**–Cu^{2+}–**H**)-(**P**–Hg^{2+}–**P**)-(**H**–Cu^{2+}–**H**)" within the double-stranded DNA was established. In light of the fact that both bases form self-complementary metallo-base pairs, it can be concluded that artificial DNAs with metallo-base pair triplets store three bits of information (i.e., $2^3 = 8$ patterns). Furthermore, a five-base-pair sequence "(**H**–Cu^{2+}–**H**)-(**H**–Cu^{2+}–**H**)-(**P**–Hg^{2+}–**P**)-(**H**–Cu^{2+}–**H**)-(**H**–Cu^{2+}–**H**)" was obtained by using a longer oligonucleotide d(5′-G**HHPHH**C-3′). In a similar fashion, consecutive alignments of heterogeneous metallo-base pairs were obtained with salen-type **Sal**–Cu^{2+}(en)–**Sal** and thymine-based T–Hg^{2+}–T pairs. Up to 10 metal-mediated base pairs were stacked within DNA duplexes, which possibly

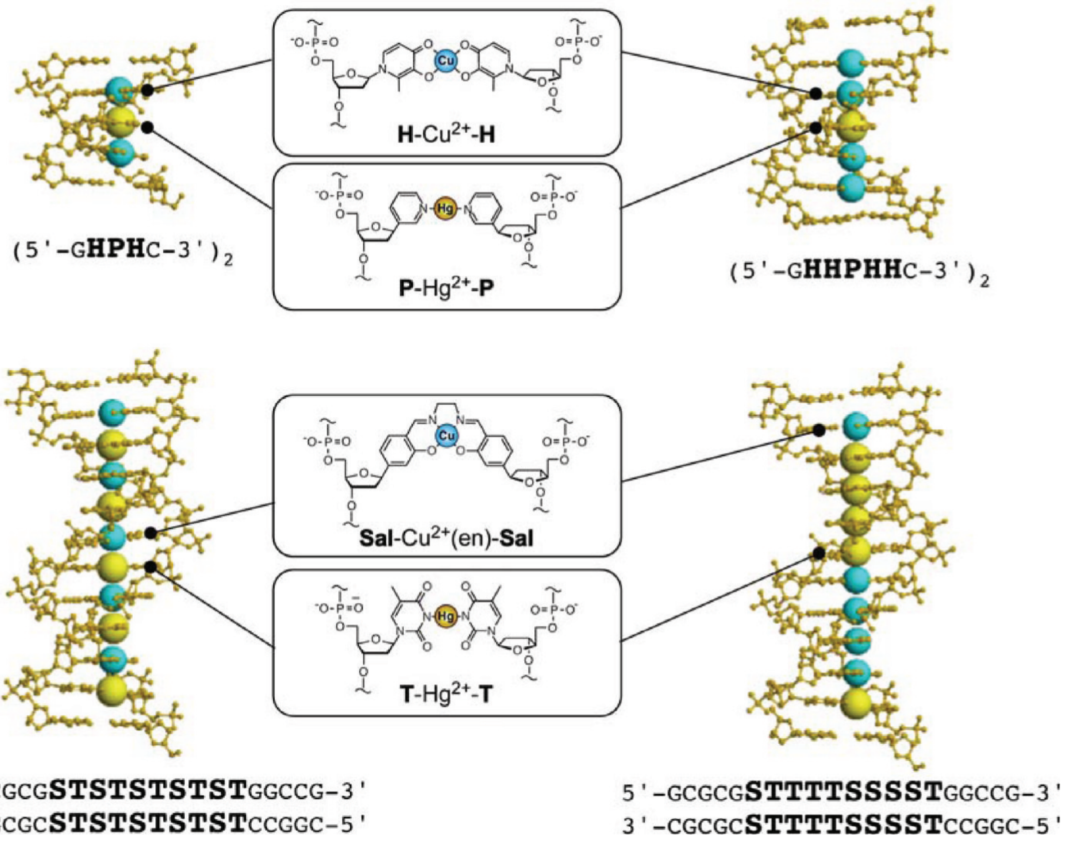

FIGURE 5. Incorporation of heterogeneous metallo-mediated base pairs. "**S**" stands for salicylaldehyde-bearing nucleoside (**Sal**). Reproduced from ref 5 (computer-generated models). Copyright 2007 Elsevier.

provides a 10-bit information storage (i.e., $2^{10} = 1024$ patterns).

In the above cases, the orthogonal metal binding affinity of the ligand-type nucleosides led to the site-selective and "full-match" metallo-base pairing. Likewise, an appropriate choice of ligand framework and metal species is crucial for generating orthogonality and complementarity of metallo-base pairing, which are essential for information storage. For more strategic designs, we employed the "hard and soft acids and bases" (HSAB) rule, that hard (soft) ligands tend to bind to hard (soft) metal ions, and synthesized two soft ligand-bearing nucleosides, mercaptopyridone-bearing nucleoside (**M**) and hydroxypyridinethione-bearing nucleoside (**S**), possessing a thiol group and a thiocarbonyl group, respectively.[36] UV-spectroscopic titration experiments and mass spectrometric measurements revealed that they form metal-mediated base pairs efficiently with square-planar, relatively soft metal ions such as Pd^{2+}, Ni^{2+}, and Pt^{2+} (**22**, **23**). More importantly, a moderate selectivity for base pairing according to the HSAB rule was confirmed by the comparison with hydroxypyridone-bearing nucleoside (**H**). This proof-of-principle demonstration would pave the way for development of orthogonal metallo-base pairs toward higher-density information storage systems using metallo-DNAs.

Incorporation of Metal-Mediated Base Pairs into Other DNA Structures

The double helix is not the only structure that can be constructed from DNA strands via interactions between nucleobases. For example, intramolecular hybridization affords hairpin loops, and recombination of more than two strands gives junction structures. Hydrogen bonding on Hoogsteen sites contributes to the formation of triple helices and quadruplexes such as G-quartet. All of them have been proven to play substantial roles in the biological systems and are now utilized as versatile structural motifs for DNA-based materials. Metallo-base pairs can be also adopted for construction of such DNA high-order structures. For example, the pyridine-bearing nucleoside (**P**), which provides a two-coordinate metallo-base pair, **P**$-Ag^+/Hg^{2+}-$**P** (**6**), in the duplex, also formed a **P**$_3Ag^+$ base triplet within a triple-stranded DNA (Figure 6A). Its stabilization effect was observed in melting temperature measurements, showing that

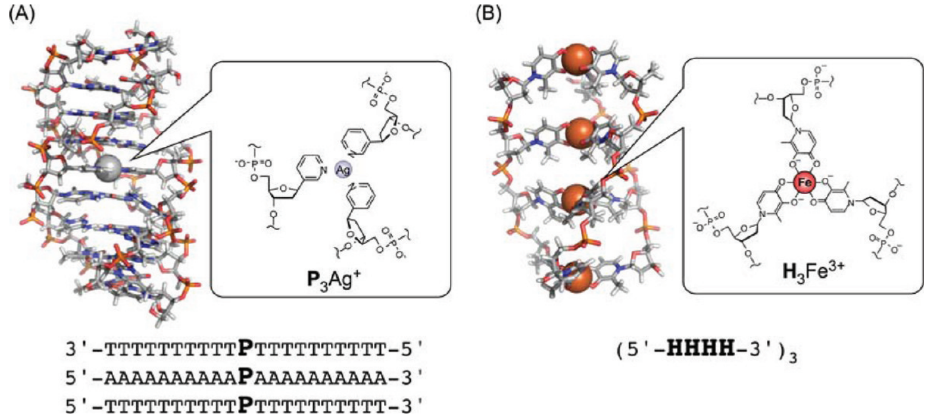

FIGURE 6. Examples of metal-mediated structural motifs leading to other DNA structures (computer-generated models): (A) $\mathbf{P}_3Ag^+$ in a DNA triplex; (B) $\mathbf{H}_3Fe^{3+}$ in a DNA triplex.

the T_m value for the third strand of the triplex d(5′-T_{10}**P**T_{10}-3′)$_2$·d(5′-A_{10}**P**A_{10}-3′) increased from 15.5 to 17.5 °C ($\Delta T_m^{metal} = +2.0$ °C) by the addition of Ag^+ ions.[37]

Induction of hairpin structures by metal-mediated base pairs has been examined due to its potential application to structural conversion of functional DNA molecules. For example, conformational changes between a hairpin structure and a double helix were demonstrated using a 1,2,4-triazole-bearing nucleoside[17] and an imidazole-bearing one (**Im**),[29] both of which form Ag^+-mediated base pairs. Without metal ions, oligonucleotides containing consecutive ligand-bearing nucleosides formed hairpin structures in which artificial nucleosides were located in their loops. In the presence of Ag^+ ions, duplexes were preferentially formed owing to Ag^+-mediated base pairing. This result established that metal-mediated base pair formation can trigger structural changes of DNA molecules.

Completely artificial helical structures were also obtained from combinations of homooligomers of hydroxypyridone-bearing nucleosides (**H**) and six-coordinate Fe^{3+} ions (Figure 6B).[38] Oligonucleotides possessing only **H** nucleosides, d(5′-$\mathbf{H}_n$-3′) ($n = 2-4$), formed triple-stranded architectures, $n$$Fe^{3+}$·d(5′-$\mathbf{H}_n$-3′)$_3$, upon complexation with Fe^{3+} ions through quantitative formation of a 3:1 $\mathbf{H}_3Fe^{3+}$ complex, so to say "metallo-base triplet".

Overall, the concept of metal-mediated base pairs can be exploited to control DNA high-order structures and has been extended to other metal-mediated structural motifs such as base triplets. In addition, the stability of the resulting DNA structures might be switched by external stimuli that affect dynamic aspects of coordination bonding. Thus, metal-mediated base pairing systems would provide highly versatile elements not only for DNA-based devices and machines but also for metal-dependent gene regulation and artificial genetic circuits.

Applications of Metallo-Base Pairs to Nanodevices

Incorporation of metallo-base pairs into DNA structures has also attracted wide interest in the field of materials science because a discrete metal line-up built up along the DNA axis is expected to afford promising nanoscale architectures applicable to molecular magnets and conductive metal nanowires. The first investigation on the magnetic property of metallo-DNAs related to the stacks of **H**–Cu^{2+}–**H** base pairs constructed inside the $n$$Cu^{2+}$·(5′-G$\mathbf{H}_n$C-3′)$_2$ ($n = 1-5$) duplexes (Figure 7A).[22] The Cu^{2+} arrays exhibited the highest spin states ($S = n/2$, $n = 1-5$) for the ferromagnetic coupling between the stacked Cu^{2+} ions through unpaired d electrons. On the other hand, the stacked **Sal**–Cu^{2+}(en)–**Sal** base pairs inside a DNA duplex were found to show predominantly antiferromagnetic coupling although their Cu^{2+}–Cu^{2+} distance is almost the same as that of the **H**–Cu^{2+}–**H** stack.[31] These results have good agreement with theoretical predictions based on density functional theory (DFT) calculations.[39]

Electric conductance of individual metallo-DNA duplexes was recently examined using a carbon nanotube device, in which a nanotube gap was covalently bridged by the DNA duplex (Figure 7B).[40] The measurement revealed that the conductance of the DNA duplexes containing one **H**–Cu^{2+}–**H** base pair was comparable to that of natural DNA duplexes, while the conductance of the ligand-containing but metal-free DNA duplexes was not measurable due to their high resistance. This indicates that the **H**–Cu^{2+}–**H** base pair favors charge transport similarly to natural

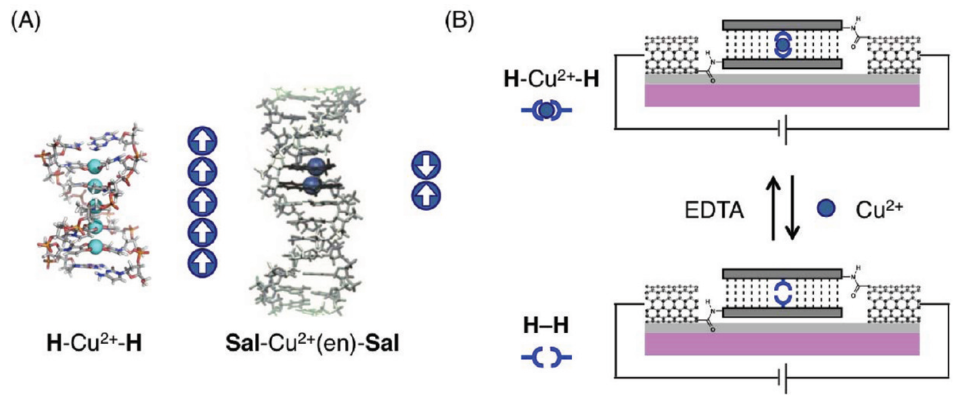

FIGURE 7. (A) Magnetic properties of Cu^{2+} complexes assembled inside artificial metallo-DNA duplexes (computer-generated models). Reproduced from ref 31. Copyright 2010 Wiley-VCH. (B) A direct conductance measurement of a single metallo-DNA duplex bridging a carbon nanotube gap.

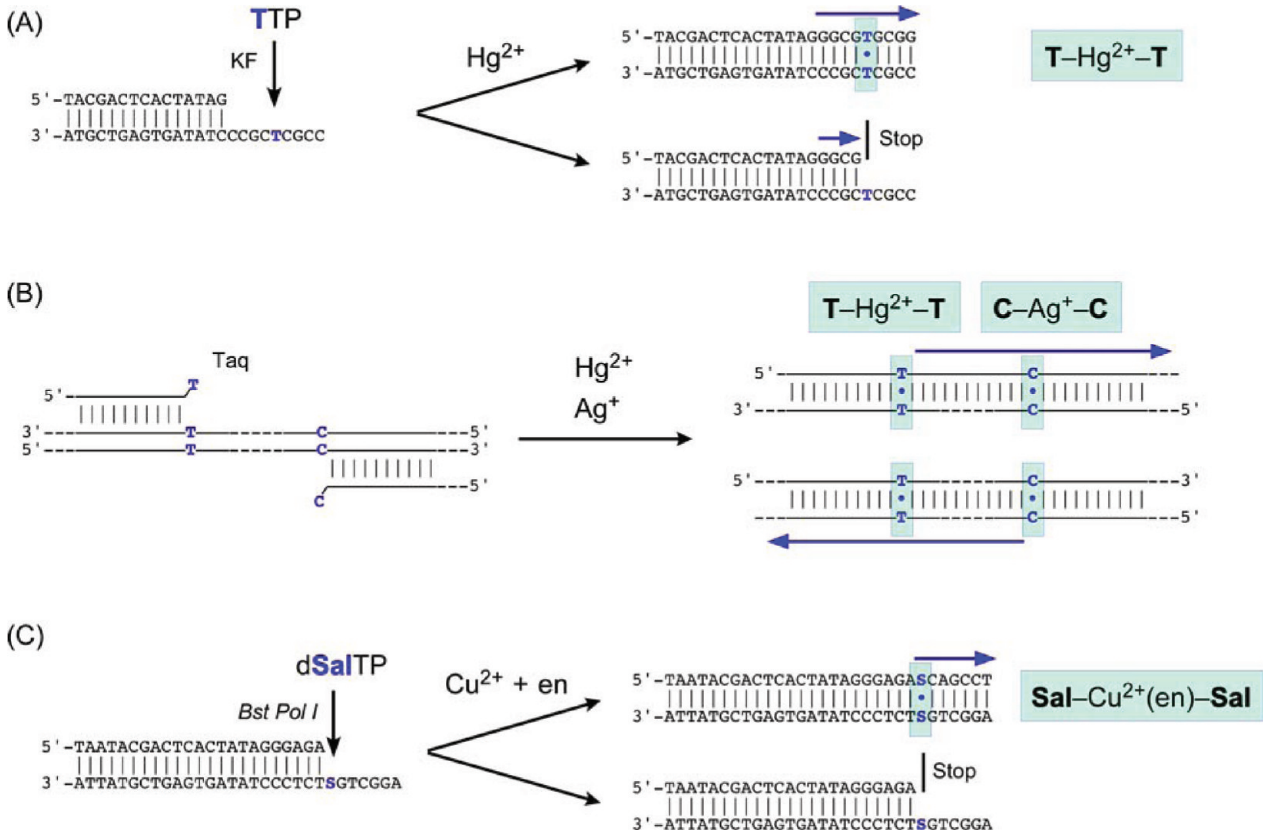

FIGURE 8. Applications of metal-mediated base pairs for enzymatic DNA polymerization: (A) Incorporation of T$-$Hg^{2+}$-$T base pairs by Klenow fragment (KF). (B) An AND logic gate system utilizing T$-$Hg^{2+}$-$T and C$-$Ag^{+}$-$C base pairs. (C) Incorporation of **Sal**$-$Cu^{2+}(en)$-$**Sal** base pairs by *Bst* DNA polymerase I. "**S**" in the sequence stands for **Sal**.

matching base pairs. Furthermore, alternate treatments of the DNA devices with Cu^{2+} ions and a chelating agent, EDTA, allowed on$-$off switching of the electrical conductance. Thus, the metallo-DNA molecule is considered to have a potential as a component of molecular-scale electrical circuits and can be applied for metal-triggered switchable devices.

Moreover, metal-mediated base pairing would allow fine-tuning of the magnetism or the electric conductivity of the metallo-DNAs, because such an approach provides a methodology to construct one-dimensional alignments of homogeneous and heterogeneous metal ions with predetermined sequences.[35] Consequently, it can be concluded that the metal-mediated base pairing systems would open a new direction in the development of molecular nanodevices, which may be interfaced with biological systems.

Applications of Metallo-Base Pairs to Enzymatic Reactions

It is safe to say that the next breakthrough regarding metal-mediated base pairs is their utilization in biological systems such as enzymatic reactions. The examination of biocompatibility and bioacceptability of the metallo-base pairs is quite primitive. The first enzymatic incorporation of metal-mediated base pairs into DNA was demonstrated with T$-$Hg^{2+}$-$T base pairs in 2010 by Urata et al. (Figure 8A).[41] Primer extension experiments showed that DNA polymerases site-selectively incorporated TTP opposite a templating thymine base only in the presence of Hg^{2+} ions, and the primers were further extended after the T$-$Hg^{2+}$-$T pair. The T$-$Hg^{2+}$-$T base pair was recently exploited in combination with the C$-$Ag^{+}$-$C pair for construction of DNA logic gates integrated with PCR amplification (Figure 8B).[14] The metal-mediated base pairs were incorporated into both primer strands and consequently PCR amplification proceeded only when both Hg^{2+} and Ag^{+} ions were added. That is, an AND gate was developed whose inputs are metal ions. In a similar way, an OR gate was also constructed with the metal-mediated base pairing. This pioneering work implies a future application of metallo-base pairs to DNA-based molecular machines and artificial genetic circuits that will surely contribute to synthetic biology.[42]

The disadvantage that these base pairs cannot be orthogonal to the native base pairs was recently overcome by adopting the **Sal**$-$Cu^{2+}(en)$-$**Sal** base pair into polymerase reactions (Figure 8C).[28] The triphosphate of salicylaldehyde-bearing nucleoside (**Sal**) was selectively incorporated opposite **Sal** on the template DNA by a *Bst* DNA polymerase I in the presence of ethylenediamine (en) and Cu^{2+} ions. Furthermore, PCR amplification of DNA strands containing three **Sal**$-$Cu^{2+}(en)$-$**Sal** base pairs was successfully performed without significant diminishment of the **Sal** content. This result suggested that the metal-mediated base pairing is a promising tool to develop a "third base pair" compatible with the canonical A$-$T and G$-$C pairs and would result in expansion of the genetic alphabet.

Conclusion

In this Account, we have overviewed the development of metal-mediated base pairs as well as their potential application to biological systems. The metallo-base pairs have been designed by selecting appropriate combinations of ligand-bearing nucleosides and metal ions mainly on the basis of their binding affinity and of their intrinsic structure. Most of them were synthetically incorporated into DNA strands without significant structural perturbation, leading to the stabilization of duplexes. This feature would be utilized to truncate functional DNAs such as aptamers and DNAzymes. Importantly, structural conversion can be triggered by the metal-mediated base pairing, which allows the construction of DNA-based switchable devices and machines that can be applied for regulation of DNA functions and for construction of synthetic genetic circuits. It is noteworthy that other properties of metallo-base pairs such as redox activity and kinetic behavior should be investigated for further applications under biologically realistic conditions. Incorporation of homogeneous or heterogeneous multiple metallo-base pairs has been also achieved, and therefore the construction of alternative information storage systems based solely on metal coordination bonding would be conceptually possible. Furthermore, enzymatic replication and amplification of the metallo-base pair have been recently demonstrated and its biocompatibility was verified. In light of the fact that metal-mediated base pairs can be designed so as to be fully orthogonal to canonical hydrogen-bonded base pairs, it is surely concluded that metal-mediated base pairing is one of the promising candidates for alternative base pairing, and further studies are expected to bring a breakthrough in construction of DNA-based intelligent molecular systems.

BIOGRAPHICAL INFORMATION

Yusuke Takezawa is Assistant Professor of the Department of Chemistry, Graduate School of Science, the University of Tokyo. He obtained his B.S. (2003), M.S. (2005), and Ph.D. (2008) from the University of Tokyo. He was also a Research Fellow of Japan Society for the Promotion of Science from 2005 to 2008. He spent one and half years as a postdoctoral fellow and a COE Assistant Professor at the Research Center for Materials Science, Nagoya University, and then he moved to Systems and Structural Biology Center, Yokohama Institute, RIKEN. In June 2010, he was appointed to his current position. His current research interest is the development of metal-modified functional DNA supermolecules.

Mitsuhiko Shionoya is Professor of the Department of Chemistry, Graduate School of Science, the University of Tokyo. He received his B.S (1982) and M.S. (1984) from the University of Tokyo. In 1986, he accepted a position as Assistant Professor at Hiroshima University. From 1988 to 1990, he obtained a position as Assistant Professor at the Institute for Molecular Science in Okazaki. He received his Ph.D. from Hiroshima University in 1990. He was appointed as Lecturer in 1991 and then as Associate Professor in 1994 at Hiroshima University. In 1991, he was a JSPS visiting scientist at the University of Texas at Austin, USA. In 1995, he was promoted to Professor at the Institute for Molecular Science in Okazaki. In 1999, he was appointed to his current position. He was a Guest Professor of University of Louis Pasteur and LMU

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FOOTNOTES

The authors declare no competing financial interest.

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