Artificial Metallo-DNA towards Discrete Metal Arrays

Mitsuhiko Shionoya

Department of Chemistry, Graduate School of Science, The University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113-0033, Japan
E-mail: shionoya@chem.s.u-tokyo.ac.jp

Summary: DNA shows promise as a provider of a structural basis for the "bottom-up" fabrication of functionalized molecular building blocks. In particular, the replacement of hydrogen-bonded DNA base pairing for alternative one could possibly provide a novel tool for re-engineering DNA as well as for biological applications. This review describes our recent approaches to metal-based strategy directed towards self-assembled metal arrays within DNAs. Recently, we reported the synthesis of a series of artificial oligonucleotides, d(5'-G$\boldsymbol{H}_n$C-3') (n = 1-5), using hydroxypyridone nucleobases ($\boldsymbol{H}$) as flat bidentate ligands. Right-handed double helices of the oligonucleotides, nCu^{2+}·d(5'-G$\boldsymbol{H}_n$C-3')$_2$ (n = 1-5), are quantitatively formed through Cu^{2+}-mediated alternative base pairing ($\boldsymbol{H}$-Cu^{2+}-$\boldsymbol{H}$), where the Cu^{2+} ions are aligned along the helix axes inside the duplexes with the Cu^{2+}-Cu^{2+} distance of 3.7 ± 0.1 Å. The Cu^{2+} ions are coupled in a ferromagnetic manner with one another through unpaired d electrons to form magnetic chains. This strategy represents a new method for self-assembled metal arrays in a predesigned fashion, leading to the possibility of metal-based molecular devices such as molecular magnets and wires.

Keywords: artificial DNA; base pairing; metal array; metal complex; metallo-DNA; self-assembly

Introduction

Research on bio-inspired molecular architecture is often directed towards the redesign of fundamental building blocks that have been provided by Nature and then the "bottom-up" syntheses of a wide range of possible structures and functions. Although biomacromolecules contain only a limited number of building blocks such as nucleotides and amino acids, owing to recent advances in chemical synthesis and biotechnology, one can replace the building blocks by chemically modified ones to arrange them one after another with a desired length and sequence.

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In addition, self-assembly protocols and template-directed procedures, which are efficiently used in the biological systems, have been conceptually introduced into chemical approaches to self-assembled, nano-sized molecules or materials. Herein we focus on the incorporation of metal complexes as alternative components into biomolecular scaffolds, which is a key design in the structural control and functionalization of biopolymers.

DNA provides a structural basis to arrange functionalized molecular building blocks into predesigned geometries. In the double-stranded DNA, hydrogen-bonded base pairs, which are attached nearly perpendicular to the phosphate backbone, are arranged into direct stacked contact. Therefore, among a variety of approaches to DNA-based supramolecular architectures, the strategy of replacing natural DNA base pairs by alternative ones possessing a distinctive shape, size, and function[1] would be expected to provide a general method of molecular arrangement within the DNA in a controllable manner. Such extra base pairs would not only expand the genetic alphabet but would also allow the replication of DNA containing unique functional groups. Moreover, DNA that is completely built out of artificial base pairs could lead to novel oligomers or polymers having unique chemical and physical properties. This review describes recent advances in artificial metallo-DNAs directed toward DNA nanotechnology as well as gene control.

Alternative Hydrogen Bonding and Non-Hydrogen Bonding Schemes for DNA Base Pairing

Watson-Crick hydrogen bonding in natural base pairs plays crucial roles in the DNA functions. Initial effort was made mainly to expand the genetic alphabet using altered hydrogen bonding. Benner and co-workers pioneered an excellent way to enzymatic incorporation of new hydrogen-bonded base pairs into DNA/RNA to extend the genetic alphabet.[2a] They reported a series of nucleobase analogs whose hydrogen-bonded patterns differ from those in the adenine-thymine (A-T) and guanine-cytosine (G-C) base pairs of natural DNA. Interestingly, even subtle changes in their hydrogen-bonding patterns have a great influence on thermodynamic[2b] and biochemical properties.[2c-e]

Watson-Crick base pairing in DNA keeps two rules of complementarity in both size and hydrogen bonding patterns. Hydrophobicity and planarity of the bases are also important for the stability of the double helical structure. In this context, Kool and co-workers have developed a series of shape mimics of natural bases lacking hydrogen bonding functionality, using the principle that two bases should be complementary in shape rather than in hydrogen bonding.[3] A set of non-hydrogen-bonding base mimics for thymine and adenine was designed based on the criteria that oxygen and nitrogen could be replaced by fluorine and carbon, respectively, keeping aromaticity intact. For example, the nucleoside bearing a difluorotoluene nucleobase is an excellent mimic of thymidine that can pair with adenine within DNAs.[3d,e] This out performing shape mimic of thymidine can also effectively substitute for thymine as the incoming substrate in the triphosphate form[4] as well as in the template strand[5a] in polymerase-related enzymatic reactions. These results overall suggest that enzymatic replication of base pairs does not need Watson-Crick hydrogen bonds as long as the components stack strongly and that shape recognition is important in the base pairing without hydrogen bonding.[5b] Others have also reported hydrophobic unnatural base pairs as attractive candidates for expansion of the genetic alphabets.[6]

Metal-Mediated Base Pairing in DNA

Basic Concept

When natural DNA bases are replaced by alternative ones that have the ability to bind metal ions, metal-mediated base pairs would be incorporated into DNAs at desired positions or could possibly be aligned along the helix axis. Since metal coordinative bond energy is intermediate between covalent and noncovalent ones, one metal-ligand bond should compensate for two or three hydrogen bonds as seen in the natural DNA base pairs. Metal ions thus incorporated would 1) stabilize high-order structures of DNA (duplex, triplex, etc), 2) allow one-dimensional metal arrays along the DNA helix axis with unique chemical and physical properties, 3) generate metal-dependent electro- or photochemical functions, 4) assemble DNA duplexes at the junctions to form two- or three-dimensional DNA networks, 5) label DNA at desired positions, and so on. Importantly, when metals are aligned into direct stacked contact within DNA duplexes, the net

charge of each metal-assisted base pair needs to be controlled so that the electrostatic repulsion between positively charged metal centers can be reduced – that is, negatively charged bases should be suitable for metal arrays.

Artificial Nucleosides Designed for Metal-Mediated Base Pairs

Recently, we have reported the first metal-assisted base pair using a *β-C*-nucleoside having a phenylenediamine base as Pd^{2+}-mediated base pairing.[7] Since then, some other *β-N*- and *β-C*-nucleosides designed for metal-mediated base pairing have been reported. Examples of artificial nucleosides having mono- to tetradentate ligands for metals so far reported are shown in Figure 1. Each nucleobase has one to four donor atoms at proper positions so that the ligand moiety can form a 1:1 to 4:1 complex with a transition metal ion in a linear, trigonal-planar, square-planar, tetrahedral, or octahedral coordination geometry. Among these geometries, a square-planar, linear, or trigonal-planar metal complex is most likely to replace a flat, hydrogen-bonded natural base pair. So far, we have reported, in addition to the above mentioned Pd^{2+}-mediated base pairing, B^{3+}-induced base paring with catechol,[8] Pd^{2+}-mediated base pairing with 2-aminophenol,[9] Ag^{+}-assisted base pairing with pyridine,[10] and Cu^{2+}-mediated base pairing with hydroxypyridone[11] as alternative base pairing modes (Figure 2). Other groups have also reported metal-mediated pairing ligand nucleobase mimics such as a Cu^{2+}-mediated base pair between pyridine and pyridine-2,6-dicarboxylate,[12a,b] an Ag^{+}-mediated pairing with 2,6-bis(methylthiomethyl)pyridine bases,[12c] and "ligandosides" using bipyridine nucleobases (Figure 2).[13]

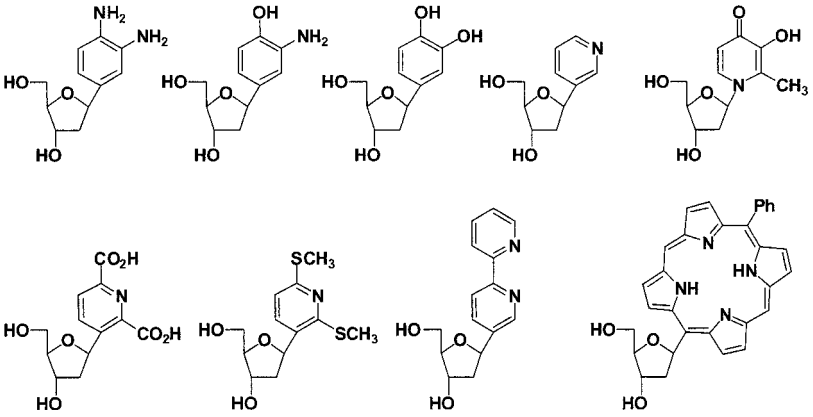

Figure 1. Examples of artificial nucleosides whose bases are replaced by metal ligands.

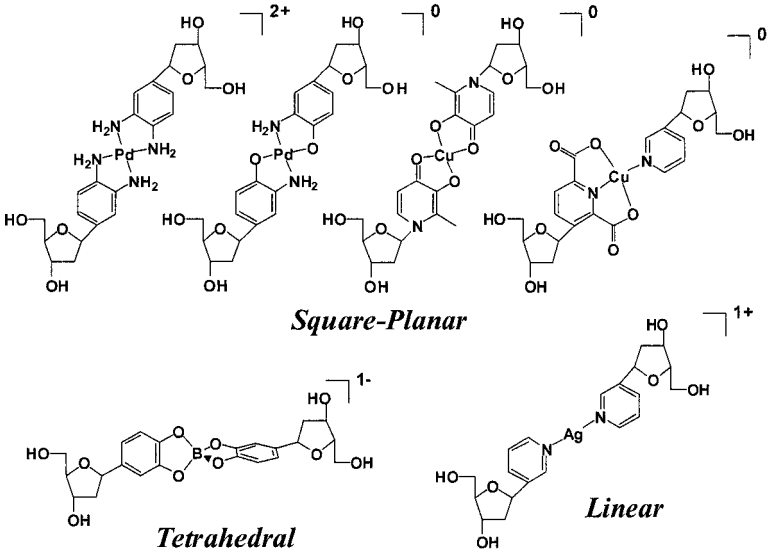

Figure 2. Examples of metal-mediated base pairs through metal-coordinating nucleoside analogues.

Single-Site Incorporation of a Metallo-Base Pair into DNA

Artificial nucleobases can be incorporated into DNA using phosphoramidite derivatives of the nucleosides with standard protocols using an automated DNA synthesizer. The first example of metal-mediated base pairing in oligonucleotides was reported by Schultz and Romesberg et al.[12a] A set of a pyridine-2,6-dicarboxylate nucleobase as a planar tridentate ligand and a pyridine nucleobase as the complementary single donor ligand was incorporated into the middle of an oligonucleotide duplex. The duplex is significantly stabilized by the formation of a neutral Cu^{2+} complex with the paired ligand bases inside the DNA.

We have independently established single-site incorporation of an Ag^+-mediated base pair into a double-stranded DNA by introducing a monodentate pyridine nucleobase in the middle of each strand.[10] For example, an Ag^+ ion incorporated into a DNA duplex, d(5'-T_{10}**P**T_{10}-3')·d(3'-A_{10}**P**A_{10}-5'), containing a pyridine nucleobase (**P**) in the middle of the sequence increases the thermal stability of the duplex due to the formation of a positively charged **P**-Ag^+-**P** base pair. In contrast, this Ag^+-dependent thermal stabilization of duplex is only slight in a reference DNA duplex, d(5'-T_{21}-3')·d(3'-A_{21}-5'). Considering the relatively weak binding between Ag^+ and pyridine in aqueous media, it appears that the **P**-Ag^+-**P** base pairing is reinforced cooperatively by the surrounding hydrogen-bonded and stacked natural base pairs in the hydrophobic environment within the duplex. Moreover, this Ag^+–mediated base pairing is specific because the addition of other transition metal ions such as Cu^{2+}, Ni^{2+}, Pd^{2+}, and Hg^{2+} showed almost no significant effects on their melting processes of the duplexes. Such thermal stabilization as seen in the case of Ag^+ is also observed with a triplex, d(5'-T_{10}**P**T_{10}-3')·d(3'-A_{10}**P**A_{10}-5')·d(5'-T_{10}**P**T_{10}-3').[10] This effect is believed to be due to the formation of a Ag^+-mediated base triplet in which the three pyridine nitrogen donors from the three strands coordinate to the Ag^+ center in a trigonal-planar coordination geometry.

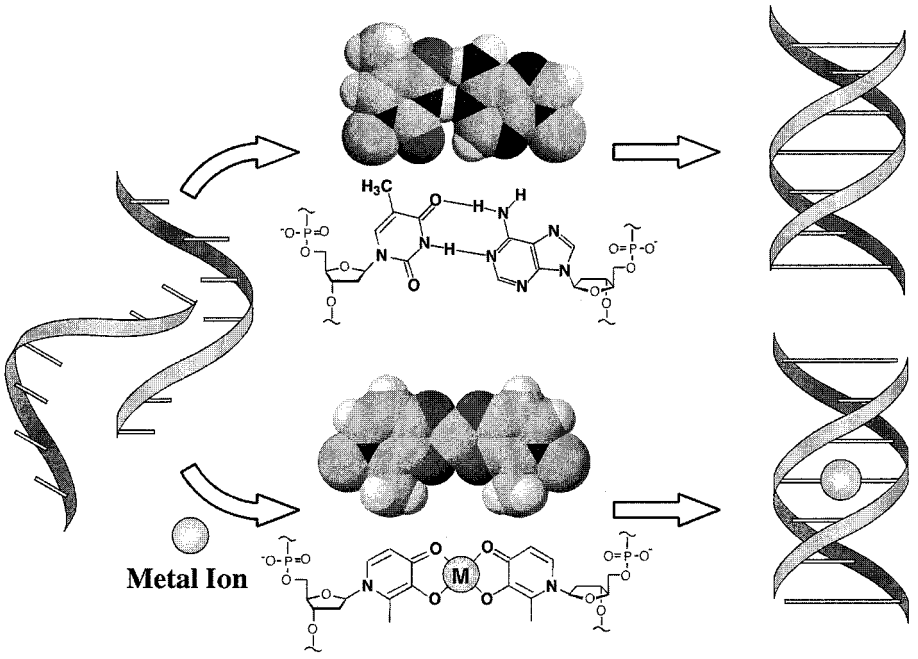

Figure 3. A schematic representation for single-site incorporation of a metal-mediated base pair.

A hydroxypiridone-bearing nucleoside was then incorporated into an oligonucleotide duplex, d(5'-CACATTA**H**TGTTGTA-3')·d(3'-GTGTAAT**H**ACAACAT-5'), to form a neutral Cu^{2+}-mediated base pair of hydroxypyridone nucleobases (**H**-Cu^{2+}-**H**) in the middle of the sequence (Figure 3).[11] In the presence of equimolar Cu^{2+} ions, an **H**-Cu^{2+}-**H** base pair is quantitatively formed within the DNA and the artificial duplex is more stabilized compared with a natural oligoduplex in which the **H**-**H** base pair is replaced by an **A**-**T** base pair. In addition, EPR and CD spectra of the metallo-DNA suggested that the radical site of a Cu^{2+} center is formed within the right-handed double-strand structure of the oligonucleotide. This strategy was further developed for controlled and periodic spacing of neutral metallo-base pairs along the helix axis of DNA.

Discrete Self-Assembled Metal Arrays in DNA

To control metal arrays at the molecular level in a discrete and predictable manner, one needs appropriate ligands having a varied number of coordination sites whose sequence can be programmable. If each metal binding site of the ligand is highly selective for some specific metal ion, the information of the sequence of the metal binding sites would be transferred into that of metals. From this standpoint, DNA, when the nucleobases are replaced by ligand-like bases, can be regarded as a multidentate ligand for one-dimensional metal arrays. In other words, the incorporation and the subsequent arrangement of metallo-base pairs into direct stacked contact within DNA could lead to "metallo-DNA" in which metal ions are lined up along the helix axis in a controlled manner.

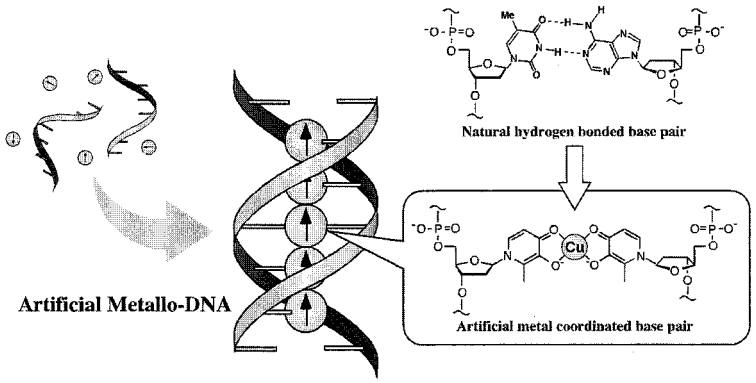

Figure 4. A schematic representation of Cu^{2+}-mediated duplex formation in which five Cu^{2+} ions are aligned along the helical axis within the DNA.

Recently, we reported the syntheses of a series of artificial oligonucleotides, d(5'-G**H**$_n$C-3') (n = 1-5), using hydroxypyridone nucleobases (**H**) as flat bidentate ligands.[14] Photometric titration studies clealy showed that right-handed double helices of the oligonucleotides, $n$$Cu^{2+}$·d(5'-G**H**$_n$C-3')$_2$ (n = 1-5), are quantitatively formed through Cu^{2+}-mediated alternative base pairing (**H**-Cu^{2+}-**H**) (Figure 4). In these metallo-DNA, the Cu^{2+} ions incorporated into each complex are aligned along the helix axes inside the duplexes with the Cu^{2+}-Cu^{2+} distance of 3.7 ± 0.1 Å as evidenced by the EPR study. The Cu^{2+} ions are coupled with one another through unpaired *d*

electrons to form magnetic chains. The electron spins on adjacent Cu^{2+} centers are aligned parallel and couple in a ferromagnetic fashion with accumulating Cu^{2+} ions attaining the highest spin state, exactly as expected from a line-up of Cu^{2+} ions. Such strategy could be developed for self-assembled metal arrays in a variety of DNA structures such as multi-stranded, hairpin, junction, or cyclic structures. Heterotopic metal arrays as well as elongation of metal strings by chemical and enzymatic methods is now underway.

Future Prospects for Artificial Metallo-DNA

Such a new binding motif in DNA duplex will influence research in areas as diverse as medicinal chemistry, materials science, and bio-nanotechnology. Introduction of metallo-base pairs into DNA would not only affect the stability of DNA double strands but also confer a variety of metal-based functions upon DNA. This strategy represents a new method for arranging metal ions in solution in a discrete and predictable fashion, leading to the possibility of metal-based molecular devices such as molecular magnets and wires. It is a real challenge to create hetero metal arrays with unique functions, leading possibly to chemical communication between different kinds of metals triggered by stimuli from outside.

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