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Metal-Mediated Base Pairs

A Highly Stabilizing Silver(I)-Mediated Base Pair in Parallel-Stranded DNA**

Indranil Sinha, Célia Fonseca Guerra, and Jens Müller**

Dedicated to Professor Stephen J. Lippard on the occasion of his 75th birthday

Abstract: *The first parallel-stranded DNA duplex with Hoogsteen base pairing that readily incorporates an Ag^+ ion into an internal mispair to form a metal-mediated base pair has been created. Towards this end, the highly stabilizing* ***6FP****-Ag^+-****6FP*** *base pair comprising the artificial nucleobase 6-furylpurine (****6FP****) was devised. A combination of temperature-dependent UV spectroscopy, CD spectroscopy, and DFT calculations was used to confirm the formation of this base pair. The nucleobase* ***6FP*** *is capable of forming metal-mediated base pairs both by the Watson–Crick edge (i.e. in regular antiparallel-stranded DNA) and by the Hoogsteen edge (i.e. in parallel-stranded DNA), depending on the oligonucleotide sequence and the experimental conditions. The* ***6FP****-Ag^+-****6FP*** *base pair within parallel-stranded DNA is the most strongly stabilizing Ag^+-mediated base pair reported to date for any type of nucleic acid, with an increase in melting temperature of almost 15 °C upon the binding of one Ag^+ ion.*

Naturally occurring DNA duplexes are composed of two antiparallel-stranded polynucleotide strands. However, parallel-stranded DNA duplexes can also be formed using canonical nucleosides.[1] Various base-pairing schemes are possible in the parallel-stranded systems, such as reverse Watson–Crick or Hoogsteen base pairing.[1,2] As the cytosine residue needs to be protonated to engage in a Hoogsteen base pair with guanine, the formation of parallel-stranded Hoogsteen duplexes can be achieved by using appropriate oligonucleotide sequences in combination with a slightly acidic reaction medium.[3]

As a result of its ability to self-assemble in a highly predictable manner and owing to its superb physical properties, DNA is being used intensively in nanobiotechnology.[4] The introduction of metal-based functionality by the formation of artificial metal-mediated base pairs (that is, the two bases of a pair are held together, not by hydrogen bonding, but by coordination to a metal center) extends this applicability even further.[5] The resulting conjugates between DNA duplexes and transition-metal complexes have been used in various manners,[6] including sensing applications, extension of the genetic alphabet,[7] and enhancing the charge-transfer capabilities of DNA.[8] Structural studies indicate that in DNA with metal-mediated base pairs the transition-metal ions are typically located inside the double helix.[9] Almost all metal-mediated base pairs reported to date have been established in the context of antiparallel-stranded DNA.[10] This is particularly true for metal-mediated base pairs involving 6-substituted purine derivatives that, in principle, should be able to form Hoogsteen base pairs in a parallel-stranded environment, too.[11] Reports on metal-mediated base pairs in parallel-stranded DNA are extremely rare:[12] DNA duplexes with reserve Watson–Crick base pairing, requiring a *transoid* orientation of the glycosidic bonds, have been applied to investigate the relative stabilities of *cisoid* and *transoid* C-Ag^+-C and T-Hg^{2+}-T base pairs (C = cytosine, T = thymine).[13] A DNA triplex has been reported in which the parallel association of the third strand to the underlying duplex is accompanied by the formation of a C-Ag^+-G:C base triple.[14] Moreover, a DNA duplex with Hoogsteen base pairing has been reported in which a terminal G:G mispair was cross-linked by a *trans*-$[Pt(NH_3)_2]^{2+}$ entity.[3] To achieve the site-specific platination, an oligonucleotide single strand containing one guanine was platinated first, purified by HPLC, and only then hybridized with the complementary strand to generate the site-specific cross-link. Finally, a few studies have been reported of duplexes comprising only metal-mediated base pairs, in which the relative orientation of the complementary strands is unknown.[15]

We report herein the first parallel-stranded DNA duplex with Hoogsteen base pairing that readily incorporates an Ag^+ ion into an internal mispair to form a metal-mediated base pair. Towards this end, we employed the artificial nucleobase 6-furylpurine (**6FP**).[16] This nucleobase can form metal-mediated homo base pairs both in regular antiparallel-stranded duplexes with Watson–Crick base pairing (with Ag^+ coordinating to N1, Scheme 1 a) and in parallel-stranded

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[*] I. Sinha, Prof. Dr. J. Müller
Westfälische Wilhelms-Universität Münster
Institut für Anorganische und Analytische Chemie & NRW Graduate School of Chemistry
Corrensstrasse 28/30, 48149 Münster (Germany)
E-mail: mueller.j@uni-muenster.de

Dr. C. Fonseca Guerra
Department of Theoretical Chemistry and Amsterdam Center for Multiscale Modeling (ACMM)
VU University Amsterdam
De Boelelaan 1083, 1081 HV Amsterdam (The Netherlands)
E-mail: c.fonsecaguerra@vu.nl

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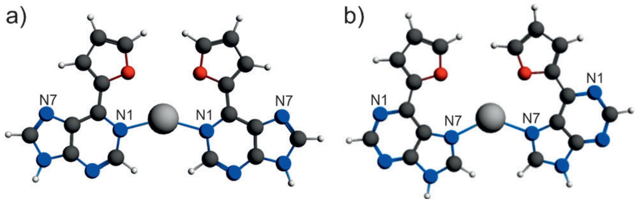


Scheme 1. Geometry-optimized structure of **6FP**-Ag$^+$-**6FP** base pairs in a) Watson–Crick-type geometry and b) Hoogsteen-type geometry.

duplexes with Hoogsteen base pairing (with Ag$^+$ coordinating to N7, Scheme 1b).

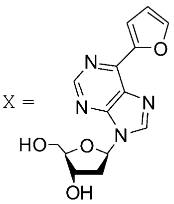
DFT calculations were performed to gain insight into the basicity and the metal-binding affinities of the various potential donor sites of **6FP**. The resulting relative proton and Ag$^+$ affinities are summarized in Table S2 (Supporting Information). As can be discerned, N1 is the preferred protonation site in the gas phase, with N7 protonation being less favored by 7.9 kcal mol^{-1}. In an aqueous environment, this difference is reduced to 2.7 kcal mol^{-1}. A similar trend is observed for Ag$^+$ binding. In this case, the N1 and N7 binding affinities are virtually identical under aqueous conditions. Hence, both base-pairing patterns depicted in Scheme 1 should be equally feasible. In all cases, the conformation in which the furyl oxygen atom is pointing towards the protonated or metalated nitrogen atom is the energetically favored one, suggesting an additional weak bonding interaction between the oxygen atom and the proton or metal ion. Interestingly, despite almost identical affinities of the two nitrogen atoms for an Ag$^+$ ion, the Ag$^+$-mediated Watson–Crick pair (Scheme 1a) is more stable than the Ag$^+$-mediated Hoogsteen pair (Scheme 1b) by 15.3 kcal mol^{-1}. This result agrees very well with recent findings for Cu^{2+}-mediated homo base pairs of 6-carboxypurine, in which the Watson–Crick geometry is more stable than the Hoogsteen geometry by 14.9 kcal mol^{-1}.[11b]

Previous investigations had shown that **6FP**:**6FP** mispairs can be incorporated into a regular antiparallel-stranded DNA duplex.[16] Upon the addition of Ag$^+$, only minor thermal stabilization of the duplex was observed. However, as the absence of an increase in the melting temperature T_m is not necessarily an indication for the lack of the formation of a metal-mediated base pair,[10a] no final conclusions regarding the formation of **6FP**-Ag$^+$-**6FP** in an antiparallel DNA duplex could be drawn at that time. The above-mentioned DFT calculations now indicate that a **6FP**-Ag$^+$-**6FP** base pair with Ag$^+$ binding to N1, that is, in the Watson–Crick geometry necessary for antiparallel duplex formation, is indeed stable (Scheme 1a). In this base pair, the distance between the N9 atoms involved in the glycosidic bonds amounts to 11.7 Å. It is larger than the distance found for the canonical base pairs (A:T, 8.89 Å; G:C, 9.01 Å). This deviation might be responsible for the fact that T_m increases only to a small extent, even though “wide” base pairs are not unprecedented in modified B-DNA duplexes.[17]

The geometry-optimized structure of the **6FP**-Ag$^+$-**6FP** base pair with Ag$^+$ binding to N7, that is, in the Hoogsteen geometry required for the formation of a parallel-stranded duplex, is stable, too (Scheme 1b, Table S3 (Supporting Information)). In this case, the distance between the N9 atoms is 6.69 Å. It is identical to that computed for Hoogsteen base pairs involving the canonical nucleobases (A:T, 6.70 Å; G:CH$^+$, 6.67 Å). This very favorable geometry suggests that the formation of such base pairs should be highly stabilizing. We therefore synthesized two oligonucleotide strands capable of forming a parallel-stranded Hoogsteen duplex under slightly acidic conditions. The sequence of this duplex incorporating one **6FP**:**6FP** mispair is given in Scheme 2. It is identical to the one used to investigate the **6FP**-Ag$^+$-**6FP** base pair in Watson–Crick geometry, albeit with a parallel orientation of the strands.

5'-d(GAG GGA XAG AAA G)-3'
5'-d(CTC CCT XTC TTT C)-3'

X =



Scheme 2. Parallel-stranded DNA duplex used in the experiments (X = **6FP**) and chemical structure of the artificial nucleoside.

Temperature-dependent UV spectroscopic investigations indicate that the parallel-stranded duplex is of low stability in the absence of Ag$^+$ ions (T_m = 15.0 °C). Upon the addition of Ag$^+$ ions, a significant stabilization is detected (Figure 1), and T_m increases to 29.5 °C in the presence of one equivalent of Ag$^+$ ions. Addition of excess Ag$^+$ ions, does not influence the melting temperature any further, indicating that a metal-mediated base pair of the type **6FP**-Ag$^+$-**6FP** is formed. To confirm independently the formation of this base pair, the absorbance of the duplex at 260 nm was plotted against the added equivalents of Ag$^+$ ions. This plot nicely illustrates that the highest affinity binding site of the duplex binds one Ag$^+$

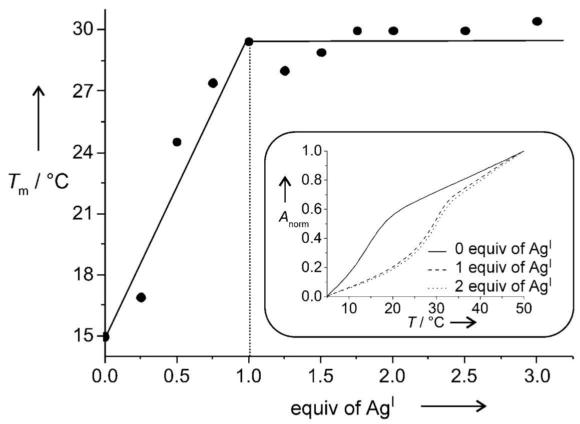


Figure 1. Increase of the melting temperature T_m in upon stepwise addition of Ag$^+$ ions to a solution of the duplex. The inset shows the melting profiles of this duplex in the presence of 0, 1, and 2 equivalents of Ag$^+$ ions, respectively.

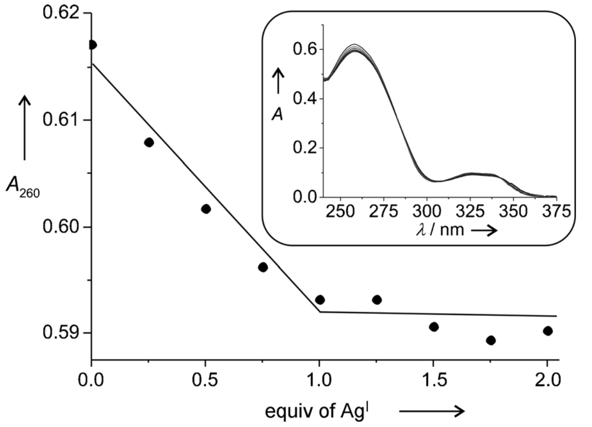


Figure 2. Plot of the absorbance at $\lambda = 260$ nm of a solution of the duplex, illustrating that one equivalent of Ag$^+$ ions is bound selectively by the duplex. Inset: the UV/Vis spectrum of the duplex in the presence of increasing amounts of Ag$^+$ ions ($0 \rightarrow 2$ equiv).

ion (Figure 2), thereby confirming the formation of the **6FP**-Ag$^+$-**6FP** base pair.

Moreover, circular dichroism (CD) spectra clearly indicate the formation of a singly metalated base pair. The formation of the **6FP**-Ag$^+$-**6FP** base pair leads to more intense Cotton effects (Figure 3), revealing a stabilization of the secondary structure. Most wavelengths of the maximum and minimum Cotton effects change only marginally. This result confirms that the overall duplex structure does not change upon insertion of the metal ion, that is, the Hoogsteen duplex remains intact in the presence of the **6FP**-Ag$^+$-**6FP** base pair. The CD spectra are clearly distinct from those of the corresponding antiparallel-stranded duplex of otherwise identical sequence, having less-intense negative Cotton effects and slightly shifted minima and maxima.[16] The positive Cotton effect at $\lambda = 343$ nm can be attributed to the presence of the furyl moiety, indicating that this group is involved in π stacking with the neighboring nucleobases and is therefore intercalated into the base pair stack. The only band that shifts significantly in the presence of Ag$^+$ ions is the negative band at $\lambda = 249$ nm. Upon the addition of Ag$^+$ ions this band shifts to $\lambda = 254$ nm and remains in this wavelength region in the presence of excess Ag$^+$ ions. Hence, the CD spectra give a further indication for the formation of the **6FP**-Ag$^+$-**6FP** base pair.

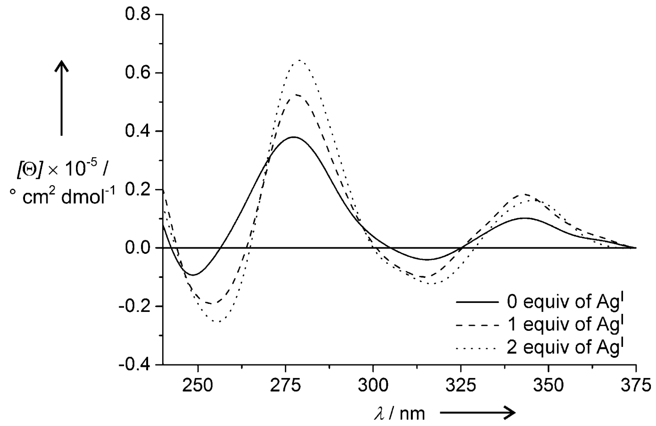


Figure 3. CD spectra of the duplex in the presence of various equivalents of Ag$^+$ ions.

To summarize, we have reported the first Ag$^+$-mediated base pair within a parallel-stranded Hoogsteen-type DNA duplex. It is formed as a homo base pair from the artificial nucleobase 6-furylpurine. This nucleobase is capable of forming both Watson–Crick-type metal-mediated base pairs (for antiparallel-stranded duplexes, Ag$^+$ binding to N1) and Hoogsteen-type metal-mediated base pairs (for parallel-stranded duplexes, Ag$^+$ binding to N7). A series of experiments as well as computations show that the **6FP**-Ag$^+$-**6FP** Hoogsteen-type base pair with parallel-stranded DNA is the most stabilizing Ag$^+$-mediated base pair reported to date, probably because the parallel-stranded DNA itself is intrinsically unstable. Hence, parallel-stranded DNA can be significantly stabilized by the formation of coordinative bonds. The substituted purine derivative **6FP** represents an excellent artificial nucleobase for the formation of metal-mediated base pairs, as different base pairs can be generated depending on the experimental conditions (e.g. oligonucleotide sequence, pH value). Owing to the large stabilizing effect that takes place upon formation of a **6FP**-Ag$^+$-**6FP** base pair, the parallel-stranded DNA reported herein will be very attractive for sensing applications based on metal-mediated base pairs.

Experimental Section

The **6FP** phosphoramidite and the oligonucleotides were synthesized as reported previously.[16] The desalted oligonucleotides were characterized by MALDI-TOF mass spectrometry (top strand: calcd for $[M+H]^+$: 4171 Da, found: 4170 Da; bottom strand: calcd for $[M+H]^+$: 3877 Da, found: 3875 Da). MALDI-TOF mass spectra were recorded on a Bruker Reflex IV instrument using a 3-hydroxypicolinic acid/ammonium citrate matrix. The UV melting experiments were carried out on a UV spectrometer CARY 100 Bio with a heating rate of 1 °C min^{-1} and a data interval of 0.5 °C. T_m values were determined from the maxima of the first derivatives of the respective melting/cooling curves. CD spectra were recorded at 10 °C on a JASCO J-815 spectropolarimeter. Experimental conditions were 3 μM oligonucleotide duplex, 500 mM NaClO$_4$, 5 mM MES (2-(N-morpholino)ethanesulfonic acid) (pH 5.5).

All calculations were performed with the Amsterdam density functional program (ADF 2013.1)[18] as reported previously.[19] Bases and base pairs were optimized at the ZORA-BLYP-D3(BJ)/TZ2P level of theory without any symmetrical constraints. The individual bases deviate only slightly from planarity and the base pair geometries were also optimized in C_s symmetry to simulate the experimental situation in the DNA stack. Comparison of the C_s and C_1 energies confirmed that the planar base pairs are not significantly higher in energy than geometrically unconstrained ones. This energy difference can easily be overcome by stacking interactions.[20] For calculations in water, solvent effects were estimated by using the conductor-like screening model (COSMO).[21]

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