Artificial Base Pairs

DOI: 10.1002/anie.200700315

An Artificial Base Pair, Mediated by Hydrogen Bonding and Metal-Ion Binding**

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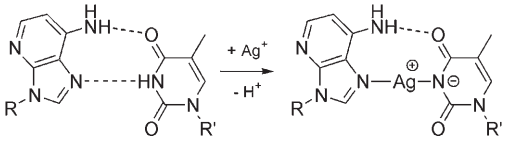
In the past years, many novel base-pairing schemes involving artificial nucleobases have been reported for nucleic acids. Several of these base pairs do not rely on hydrogen bonding between the complementary nucleobases, instead stabilization occurs through the incorporation of metal-ions into the base pair.[1–6] Recent work includes nucleic acids with up to ten consecutive metal-mediated base pairs.[1a,d] Even the defined arrangement of two different metal ions is possible.[1a] The motivation for the incorporation of metal ions into oligonucleotides is based on the anticipated chemical and physical properties of the products and their potential applicability as molecular wires in self-assembling electronic circuits.[7] Typically, the artificial nucleobase acts as a bi- or tridentate ligand which, in the presence of appropriate metal ions, leads to a significant thermal stabilization of the DNA duplex. In conjunction with the increased stability of the base pair, it is possible that the ability of the DNA strands to reversibly self-assemble decreases.[7c] It is therefore desirable to develop a base pair that is only slightly more stable than a natural one but still contains a metal ion.

Herein we report on a type of base pair that is mediated by hydrogen bonding *and* metal-ion binding. This approach combines the reliable self-recognition of complementary strands by hydrogen bonding with an increased stability arising from coordination-bond formation.[8] To this end, we chose thymine (T) and the artificial 1-deazaadenine (D) as complementary bases. Through the substitution of the N1-position of adenine by a CH group, Watson–Crick pairing is not possible between D and T. Instead, it had been shown that the oligonucleotides $d(D_{20})$ and $d(T_{20})$ associate through the Hoogsteen edge of 1-deazaadenine to form a double helix.[10] Our idea was to substitute the thymine amide proton by a metal ion (Scheme 1). This substitution is not expected to lead to a disruption of the remaining hydrogen bond. In the related complex *trans*-$[Pt(NH_3)_2$(9-MeA-*N7*)(1-MeT-*N3*)$]^+$ consisting of 9-methyladenine (9-MeA) and 1-methylthymine (1-MeTH), this hydrogen bond is still detected.[11] A geometry optimization by DFT methods of a system comprising 9-methyl-1-deazaadenine, 1-methylthyminate, and one Ag^+ ion also suggests the formation of the H bond in our system (see Supporting Information). For our experiments, we used Ag^+ ions as they prefer to have a linear coordination geometry and form stable complexes with nitrogen donor ligands. Furthermore, there are several examples of Ag^+-ion coordination compounds with short metal–metal distances resulting from stabilizing argentophilic interactions,[12] these distances nicely match the distance between neighboring base pairs in DNA.



Scheme 1. Proposed formation of a base pair mediated by an Ag^+ ion and a hydrogen bond (R, R′ = 2′-deoxyribose of a DNA backbone).

Initial experiments using an equimolar mixture of the oligonucleotides d(ADADADADA) and $d(T_9)$[13] showed promising results: In the absence of Ag^+ ions, the temperature-dependent plot of the absorbance at 260 nm does not show any cooperative melting. Upon addition of Ag^+ ions, however, distinct melting curves are obtained, with a melting temperature T_m of 18 °C (see Supporting Information). The appearance of the melting curves changes until about one equivalent of Ag^+ ion is present, that is, until all the D–T base pairs are mediated by Ag^+ ions.[14] The individual component strands do not exhibit cooperative melting under identical conditions. Interestingly, it appears that the Ag^+ ions do not insert into A–T base pairs, but only into D–T base pairs. Because of the relatively low melting point of this system, a meaningful characterization is difficult. We therefore chose to investigate the more stable duplex formed from $d(D_{19}A)$ and $d(T_{20})$. Again, no cooperative melting was observed in the absence of Ag^+ ions (Figure 1). Previous measurements using $d(D_{20})\cdot d(T_{20})$ gave a T_m of 15 °C.[10] However, those experiments had been performed with higher salt and oligonucleotide concentrations,[15] so that for $d(D_{19}A)\cdot d(T_{20})$ under our conditions a T_m of less than 15 °C is not unexpected. Upon addition of increasing amounts of $AgNO_3$, distinct melting curves are observed. The melting temperature increases steadily until one equivalent of Ag^+ ion is present. Under these conditions, T_m reaches a value of 51.2 °C, corresponding to an increase of more than 36 °C, or approximately 2 °C per base pair. An excess of Ag^+ ion does not lead to further stabilization (Figure 1). Clearly, the duplex reaches its maximum stability at a stoichiometric ratio where every amide proton can be substituted by one Ag^+ ion. This

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[**] Financial support from the DFG (J.M.; Emmy Noether program), the FCI and the Department of Chemistry at the University of Dortmund is gratefully acknowledged. We thank T. van der Wijst for performing the DFT calculations. J.M. thanks Prof. Dr. Bernhard Lippert for his continuous support.

Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

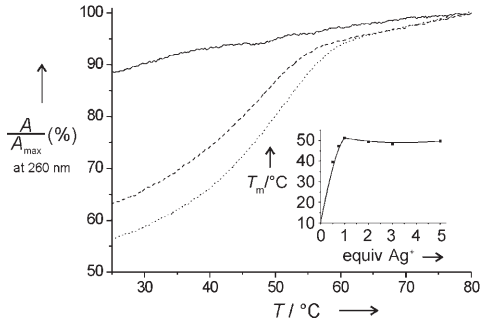

Figure 1. Melting behavior of $d(D_{19}A)\cdot d(T_{20})$ in the absence (——) and presence of different amounts of $AgNO_3$ ((-----) 0.75 equiv; (•••••) 1 equiv). The hyperchromicity changes from 11% to 44% upon addition of 1 equivalent of Ag^+ ions. Conditions: 1 μM $d(D_{19}A)\cdot d(T_{20})$, 1 M $NaClO_4$, 5 mM MOPS buffer (pH 6.8). Inset: melting point of $d(D_{19}A)\cdot d(T_{20})$ in the presence of different equivalents of $AgNO_3$. The T_m in the absence of Ag^+ ions is estimated based on ref. [10].

conclusion is corroborated by the observation, that the UV spectrum of $d(D_{19}A)\cdot d(T_{20})$ changes upon addition of $AgNO_3$ until one equivalent of Ag^+ ion is present (Figure 2). An

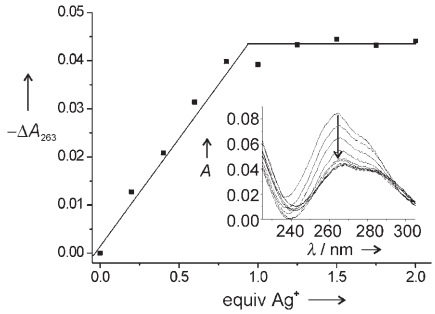

Figure 2. UV absorption changes at 263 nm of $d(D_{19}A)\cdot d(T_{20})$ upon addition of $AgNO_3$. Inset: UV spectra of $d(D_{19}A)\cdot d(T_{20})$ with various amounts of $AgNO_3$. The arrow indicates the direction of the changes.

excess of Ag^+ ion does not lead to any further changes. That this region of the spectrum is affected by Ag^+-ion binding clearly shows that the metal ion coordinates to the nucleobase, and that it is not metal binding to the backbone that causes the increase in T_m.

In a series of control experiments, the melting behavior of the individual component strands has been analyzed (see Supporting Information). The purine strand does not show any cooperative melting, irrespective of the Ag^+-ion concentration. The pyrimidine strand on the other hand reversibly forms some high-melting species in the presence of Ag^+ ions. Because thymine forms mercury(II)-mediated homo base pairs,[6] we attribute our finding to the formation of analogous silver(I)-mediated base pairs. The T_m values observed for $d(T_{20})$ do not match those of the $d(D_{19}A)\cdot d(T_{20})$ system, and neither does the T_m level off when a certain concentration of Ag^+ ion has been reached. Taken together, these data suggest that the well-defined species formed from $d(D_{19}A)\cdot d(T_{20})$ upon addition of Ag^+ ions is not the same as those that originate from $d(T_{20})$ and Ag^+ ions.

To gain insight into the conformation of the duplex $d(D_{19}A)\cdot d(T_{20})$, circular dichroism (CD) spectroscopic measurements were performed. The CD spectrum of $d(D_{19}A)\cdot d(T_{20})$ is clearly distinct from the sum of the spectra of its components $d(D_{19}A)$ and $d(T_{20})$ (Figure 3). This result shows

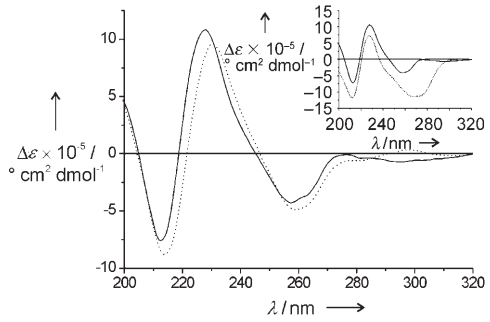

Figure 3. CD spectra of $d(D_{19}A)\cdot d(T_{20})$ in the absence (——) and presence of 1 equivalent of $AgNO_3$ (•••••). Inset: CD spectra of $d(D_{19}A)\cdot d(T_{20})$ (——) and the sum of the CD spectra of the individual strands $d(D_{19}A)$ and $d(T_{20})$ (•••••). Conditions: 3 μM $d(D_{19}A)\cdot d(T_{20})$, 1 M $NaClO_4$, 5 mM MOPS buffer (pH 6.8), 25 °C.

that under the conditions of the CD experiments (threefold higher oligonucleotide concentration than the UV melting experiments), the duplex is stable at room temperature.[16] Addition of the first equivalent of $AgNO_3$ leads to small but significant changes in the CD spectrum (Figure 3). The minima at 213 and 256 nm as well as the maximum at 226 nm experience a bathochromic shift of about 3 nm. Furthermore, a broad maximum appears at 299 nm. This latter change occurs in the region of the CD spectrum that is influenced most by base pairing and cross-strand interactions.[17] This feature thus indicates a subtle structural change inside the duplex, such as the replacement of a proton by an Ag^+ ion. The scenario is supported by the fact that the overall appearance of the CD spectrum does not change much, because no major conformational changes are expected upon substitution of H^+ by Ag^+ ions. Addition of an excess of Ag^+ ion to $d(D_{19}A)\cdot d(T_{20})$ does not lead to any further spectroscopic changes (see Supporting Information). Again, this observation is strong evidence for specific binding of one Ag^+ ion per base pair.

Interestingly, the de- and renaturing profiles recorded during the melting experiments superimpose up to one equivalent of added $AgNO_3$, whereas when an excess of Ag^+ ion is present the melting temperatures of the renaturing profiles are reproducibly higher than those of the denaturing profiles (Supporting Information). We attribute this result to the kinetically preferred formation of an intermediate hairpin structure of $d(T_{20})$ involving metal-mediated $T\cdots Ag^+\cdots T$ base pairs upon slow cooling of $d(D_{19}A)$, $d(T_{20})$, and Ag^+ ions, in accord with the formation of such base pairs from $d(T_{20})$ and Ag^+ ions alone (see above). This assumption is in agreement with the ability of Ag^+ ions to deprotonate uridine (U) and poly(U) even under slightly acidic conditions.[18,19] These hairpin structures then dissociate again to form the hetero duplex $d(D_{19}A)\cdot d(T_{20})\cdot Ag^+$ at lower temperatures, as is clear

from the similarity of the CD spectra of $d(D_{19}A)\cdot d(T_{20})$ in the presence and absence of Ag^+ ions.

To summarize, we have presented evidence for the formation of metal-mediated base pairs that also comprise a hydrogen bond. Specific binding of one Ag^+ ion per base pair leads to the formation of $d(D_{19}A)\cdot d(T_{20})\cdot Ag^+$, accompanied by an increase of the melting temperature of more than 36 °C. Base pairs of this type combine the reliable self-recognition of complementary strands based on hydrogen bonding with the increased stability of coordination bonds. Therefore, the corresponding oligonucleotides are a addition to the repertoire of structural elements available for DNA assembly in DNA nanotechnology.

Experimental Section

The preparation of the 1-deaza-2′-deoxyadenosine derivative suitable for automated DNA synthesis was carried out as described elsewhere.[10] The oligonucleotides containing 1-deaza-2′-deoxyadenosine were prepared using a Beckman Oligo 1000 M synthesizer, purified by HPLC with a Nucleogen 60-7 DEAE column, desalted with NAP 10 columns, and their identity was confirmed by MALDI-TOF mass spectrometry (d(ADADADADA): calcd for $[M+H]^+$: 2754, found: 2756; $d(D_{19}A)$: calcd for $[M+H]^+$: 6184, found: 6191). The oligonucleotides $d(T_{20})$ and $d(T_9)$ were purchased from Eurogentec. The reported T_m values correspond to the maximum values of the first derivatives of the melting curves.

Received: January 23, 2007
Revised: May 2, 2007
Published online: June 22, 2007

Keywords: bioinorganic chemistry · DNA · Hoogsteen pairs · hydrogen bonds · nucleobases

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[8] A related base-pairing pattern has been proposed for so-called M-DNA, a DNA conformation that is formed at elevated pH values in the presence of appropriate metal ions.[9a] However, the precise structure of M-DNA remains controversial.[9b]

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[13] In the case of guanine and cytosine, the formation of Hoogsteen base pairing requires a low pH value to ensure protonation of cytosine. As a low pH value impedes deprotonation of thymine, which is a prerequisite for the substitution of the amide proton by a metal ion, only oligonucleotides containing adenine, 1-deazaadenine, and thymine are considered herein.

[14] One equivalent of Ag^+ means one Ag^+ ion per D–T base pair.

[15] We could not use the conditions of the original measurements because the buffer that was used is incompatible with the presence of silver(I).

[16] The CD spectrum of $d(D_{19}A)\cdot d(T_{20})$ in the presence of 3 equivalents of Ag^+ ions has been recorded at 25 °C and 10 °C. The fact that both spectra are identical (Supporting Information) proves that cooling the sample to below 25 °C does not induce any structural changes.

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[19] We confirmed that this is also true for $d(T_{20})$ by observing a drop in pH value upon addition of one equivalent of Ag^+ ions to an unbuffered solution of $d(T_{20})$.

