Inorg. Chem. **2007**, *46*, 10114–10119

Conformational Change Induced by Metal-Ion-Binding to DNA Containing the Artificial 1,2,4-Triazole Nucleoside

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Received May 8, 2007

A conformational switch can be induced upon the addition of transition-metal ions to oligonucleotides that contain a row of successive artificial nucleobases flanked by complementary sequences of natural nucleobases, provided that the artificial bases cannot undergo self-pairing via hydrogen bonding but only via the formation of metal-ion-mediated base pairs. Such oligonucleotides adopt a hairpin structure in the absence of transition-metal ions, yet they show a preference for the formation of a regular double helix if the appropriate metal ions are present. We report here our experimental data on the structure of the oligonucleotide $d(A_7X_3T_7)$ (A = adenine, T = thymine, X = 1,2,4-triazole) in the absence and presence of silver(I). This study comprising temperature-dependent UV spectroscopy, CD spectroscopy, MALDI-TOF measurements, fluorescence spectroscopy, and dynamic light scattering opens up a new approach to the generation of a large variety of metal-ion sensors with the possibility of fine-tuning their sensing capabilities, depending on the artificial nucleoside that is used.

Introduction

Research on nucleic acids containing modified nucleosides used to be the purview of organic chemists only. Recently introduced artificial nucleosides are now also attracting inorganic chemists to this field: In nucleic acids comprising these novel nucleosides, complementary nucleobases no longer pair via hydrogen bonding but via coordinative bonds to a metal ion that is located in the middle of the double helix. Hence, the artificial nucleobases serve as ligands for the metal ions. Several examples are known to date of deoxyribonucleic acids (DNA)[1−10] and peptide nucleic acids (PNA)[11] that contain such metal-ion-mediated base pairs. A few model studies have also appeared in the past years.[12−14] These bioinspired constructs have sparked great interest due to their expected interesting chemical and physical properties such as electrical conductivity or magnetism.[10,15] The ability to create defined arrays of (different) metal ions in a highly controllable manner in a chiral environment may also be applied in asymmetric catalysis.[16] The generation of DNA-based sensors for mercury(II) ions or for the redox environment has also been reported, making use of the high affinity of the natural nucleoside thymidine toward Hg^{2+} ions.[8c,17]

We have recently reported on the potential use of artificial azole nucleosides (azole = imidazole, 1,2,4-triazole, tetra-

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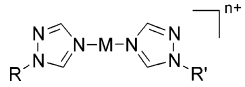
10.1021/ic700884q CCC: $37.00
Published on Web 11/01/2007

Scheme 1. Metal-Ion-Mediated Base Pair Formed from 1,2,4-Triazole Nucleoside (R, R′ = Deoxyribose)



zole) for the generation of metal-ion-mediated base pairs (Scheme 1).[12b,c]

Out of these nucleosides, the one carrying 1,2,4-triazole as a nucleobase appears particularly interesting, as it is able to discriminate silver(I) against mercury(II). ^{1}H NMR spectroscopy studies had suggested that 1:1 complexes are formed with Hg^{2+}, whereas the 2:1 stoichiometry of the nucleoside and metal ion that is necessary for the formation of a metal-ion-mediated base pair is adopted by the Ag^+ complex.[12b,c] Hence, oligonucleotides containing 1,2,4-triazole nucleoside should also show a different response to these linearly coordinating metal ions. We therefore set out to investigate the applicability of artificial nucleosides for the generation of a metal-ion sensor (using 1,2,4-triazole nucleoside as an example) whose sensing capability could be tuned depending on the type of nucleoside that is being used.

Experimental Section

1,2,4-Triazole-*N1*-2′-deoxyribonucleoside and its derivatives suitable for automated DNA synthesis were prepared according to published protocols;[12c,18] all of the other phosphoramidites and CPGs were purchased from Glen Research. The oligonucleotides were prepared using either a Beckman Oligo 1000M synthesizer or an Expedite 8909 machine in the DMT-off mode and purified by HPLC with a Nucleogen 60−7 DEAE column. The following linear gradients were used during HPLC purification: 0−5 min: 100% A, 5−20 min: 100−83% A, 20−30 min: 83−76% A (1.5 mL/min, solvent A: 20 mM sodium acetate (adjusted to pH 6 by means of acetic acid) + acetonitrile (4:1), solvent B: solvent A plus 2 M lithium chloride). After desalting via NAP 5 columns, the identity of the oligonucleotides was confirmed by MALDI-TOF mass spectrometry on Perseptive Biosystems Voyager and Bruker Autoflex II instruments (d($A_7X_3T_7$): Calcd for $(M+H)^+$: 5002, found: 5000; d($A_7X_4T_7$): Calcd for $(M+H)^+$: 5249, found: 5252; **A**: Calcd for $(M-2H+Na)^-$: 5589, found: 5589; **B**: Calcd for $(M-2H+Na)^-$: 5453, found: 5442; **C**: Calcd for $(M-2H+K)^-$: 6036, found: 6034). Unmodified oligonucleotides were purchased from Eurogentec. Prior to all of the experiments with oligonucleotides, the solutions have been heated to approximately 70 °C and then slowly cooled down to ambient temperature to ensure a homogeneous hybridization. Oligonucleotide solutions contain 150 mM $NaClO_4$ and 5 mM MOPS (adjusted to pH 6.8), unless specified otherwise. Melting temperatures T_m have been determined by monitoring the temperature dependence of the absorbance at 260 nm (heat rate: 1 °C/min, data collection: 0.2 °C/min) with a Varian CARY 100 BIO spectrometer. The reported T_m values correspond to the temperatures at the maximum values of the first derivatives of the melting curves. CD spectra were recorded on a JASCO 810 instrument. Fluorescence spectra were recorded on a Varian CARY Eclipse spectrophotometer at an oligonucleotide concentration of 20 nM with an excitation wavelength of 480 nm. Dynamic light scattering (DLS) measurements were performed with a DynaPro Titan Ambient MicroSampler at oligonucleotide concentrations of 0.2−0.5 mM. Prior to the DLS measurements, the samples were centrifuged at 13 400 rpm for 40 min. In all of the measurements, the polydispersity was around 7.5%, showing that all of the samples were monomodal monodispers. The sample containing silver(I) was prepared as follows: Addition of 2 equiv. of $AgNO_3$, heating the solution to approximately 70 °C and slowly cooling it down again, addition of Chelex 100 to remove excess silver(I), shaking for 1 h, centrifuging to remove the Chelex, rinsing the Chelex with water to recover any remaining DNA, and adjusting the pH to 6.8.

Results and Discussion

Having in mind the generation of an oligonucleotide-based metal-ion sensor, we have incorporated the artificial 1,2,4-triazole nucleoside (denoted X) into the strands d($A_7X_3T_7$) and d($A_7X_4T_7$) (A = adenine, T = thymine). The sequences were chosen in a way that the oligonucleotides should form a hairpin structure in the absence of any transition-metal ions because of the lacking capability to engage in hydrogen bonding. Addition of the appropriate metal ions should stabilize a regular double helix with metal-ion-mediated base pairs, leading to a conformational change (Scheme 2) that could be used to sense the metal ion under investigation. A similar effect had already been reported for the natural nucleoside thymidine with respect to mercury(II).[9]

In a first step, the melting points of the oligonucleotides were determined by UV spectroscopy depending on their respective concentrations. As hairpins display unimolecular melting behavior, a concentration-independent melting tem-

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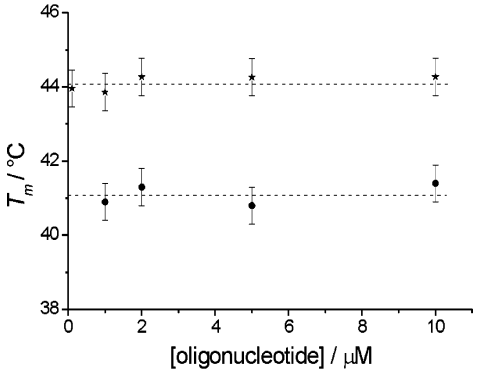


Figure 1. Concentration-independent melting temperatures of d($A_7X_nT_7$) (★, $n = 3$; ●, $n = 4$) confirm the presence of hairpin structures in the absence of transition-metal ions.

Scheme 2. Conformational Change from Hairpin to Regular Double Helix Induced by the Addition of Appropriate Metal Ions (Gray Spheres) Due to the Stabilizing Effect of Metal-Ion-Mediated Base Pairs

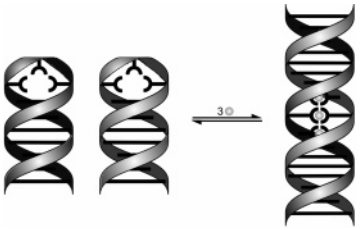


Table 1. Oligonucleotides with Appended Florescence Donor and/or Quencher Moieties

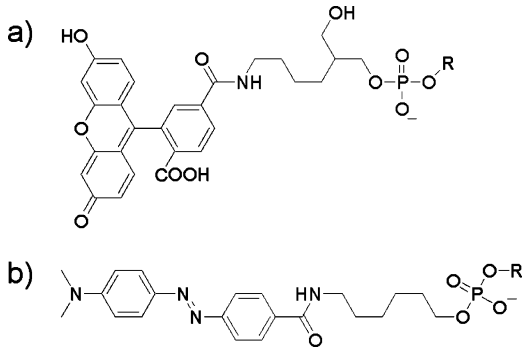
	sequence
A	5′-d($A_7X_3T_7$)-3′-fluorescein
B	dabcyl-5′-d($A_7X_3T_7$)-3′
C	dabcyl-5′-d($A_7X_3T_7$)-3′-fluorescein

perature T_m would confirm the presence of this conformation.[19] As can be seen from Figure 1, both oligonucleotides d($A_7X_nT_7$) with $n = 3$, 4 adopt a hairpin structure. As a result of its higher thermal stability, the sequence d($A_7X_3T_7$) was chosen for all further experiments.

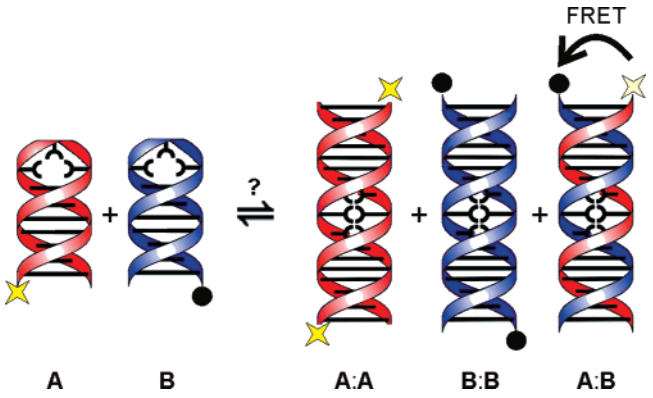
To verify independently the formation of a hairpin structure in the absence of transition-metal ions, we synthesized various oligonucleotides derived from d($A_7X_3T_7$) with an appended fluorescence donor and/or quencher moiety (Table 1). 6-Fluorescein was chosen as a donor and dabcyl as a quencher molecule (Scheme 3). In an equimolar mixture of oligonucleotides **A** and **B**, fluorescence should not be quenched if a hairpin conformation is adopted by the nucleic acids because fluorophore and quencher are never present within the same molecule. In case of the formation of double helices, however, three different duplexes are expected to be present in solution: **A**:**A**, **B**:**B**, and **A**:**B** (Scheme 4).

In adduct **A**:**B**, which is expected to form with a probability of 50%, the fluorescein and dabcyl moieties are located at close distance from each other, hence fluorescence resonance energy transfer (FRET) should lead to efficient quenching of the fluorescence. As can be seen from Figure 2, no such quenching is observed in an equimolar mixture

Scheme 3. Structures of the (a) 6-Fluorescein and (b) Dabcyl Labels Used in Oligonucleotides **A**, **B**, and **C** (R = Oligonucleotide)



Scheme 4. Principle of How to Differentiate between Hairpin and Double Helix in an Equimolar Mixture of **A** and **B** by Measuring the Fluorescence of the Solution



of **A** and **B**. Therefore, these nucleic acids must adopt a hairpin conformation. The above-mentioned quenching due to FRET is observed for oligonucleotide **C**, in which the fluorophore and quencher are at a close distance, irrespective of the nucleic acid conformation.

We then set out to investigate the influence of transition-metal ions on the stability of the oligonucleotides. Because of steric requirements, only linearly coordinating metal ions such as Ag^+ or Hg^{2+} can be incorporated into azole-based metal-ion-mediated base pairs.[12b,c] Previous work had suggested that 1,2,4-triazole nucleoside forms 2:1 complexes with silver(I), which is in line with the formation of a base pair, whereas 1:1 complexes are formed with mercury(II).[12b,c] We therefore examined the effect of these two metal ions on the melting temperature of d($A_7X_3T_7$). Not unexpectedly, the addition of Hg^{2+} to the oligonucleotide leads to a destabilization (Supporting Information, Figure S1), which could be due to the preferential formation of a 1:1 adduct with the artificial nucleobase.[20] The addition of Ag^+, however, has a large stabilizing effect on the oligonucleotide. Interestingly, the T_m values are now dependent on the oligonucleotide concentration (Figure 3).

Hence, the melting behavior is no longer unimolecular, which is a first indication that the rearrangement toward a regular double helix takes place.[19] When plotting the stability increase against the equivalents of added Ag^+ ions (as in

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(20) Because of the high affinity of thymidine towards Hg^{2+} (refs 8 and 9), the undesired formation of such adducts might also be responsible for the observed destabilization.

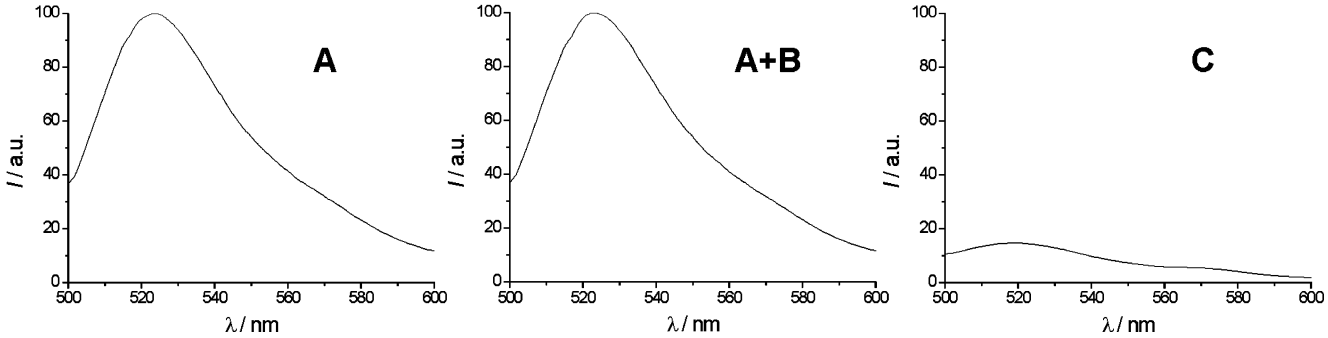

Figure 2. Fluorescence spectra of oligonucleotide **A**, of an equimolar mixture of **A** and **B**, and of **C**.

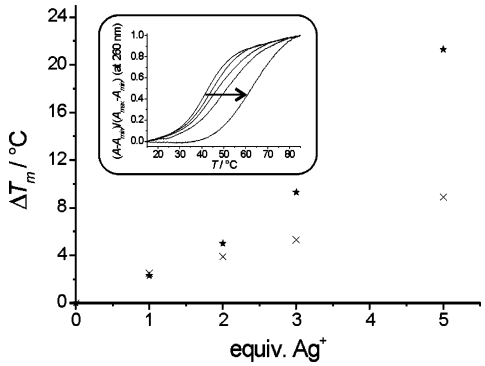

Figure 3. Increase in melting temperature ΔT_m of d($A_7X_3T_7$) upon the addition of increasing amounts of $AgNO_3$. Conditions, n μM oligonucleotide (×, $n = 1$; ★, $n = 3$), 150 mM $NaClO_4$, 5 mM MOPS pH 6.8. Inset: Melting curves of 3 μM d($A_7X_3T_7$), from left to right: 0, 1, 2, 3, and 5 equiv of $AgNO_3$.[21]

Figure 3),[21] a higher oligonucleotide concentration automatically translates into a higher silver(I) ion concentration. To make sure that the observed increase in stability is not merely an effect of this increased silver(I) concentration, the experimental data also need to be plotted against the Ag^+ concentration (part a of Figure 4). This plot clearly shows that it is not solely the Ag^+ concentration that influences the melting temperature. The different slopes found for the two oligonucleotide concentrations suggest that the metal ions bind specifically at the desired locations, that is, in between the complementary triazole nucleosides.[22] Identical slopes for varying concentrations of nucleic acid on the other hand would be expected if nonspecific binding took place. Indeed, such behavior is found for the sequence d(A_7-$GAAT_7$), which contains the natural nucleobases guanine and adenine instead of the artificial ones (part b of Figure 4). The GAA sequence is well-known to stabilize hairpin structures.[23]

Additional experiments were performed to gain further insight into the changes in oligonucleotide conformation by

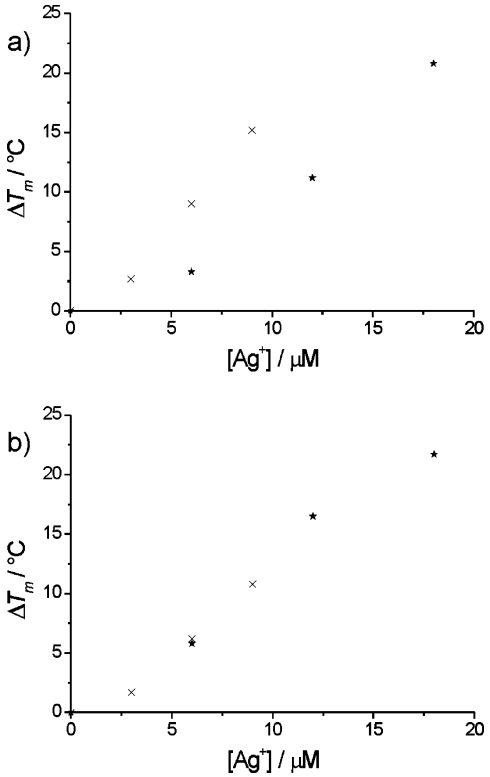

Figure 4. Increase in melting temperature ΔT_m of (a) modified oligonucleotide d($A_7X_3T_7$) and (b) unmodified oligonucleotide d(A_7GAAT_7) upon the addition of increasing amounts of $AgNO_3$. Conditions, n μM oligonucleotide (×, $n = 1$; ★, $n = 2$), 150 mM $NaClO_4$, 5 mM MOPS pH 6.8.

applying CD and UV spectroscopy. The CD spectra of d($A_7X_3T_7$) show a steady transition upon increasing silver(I) concentrations (Supporting Information, Figure S3). At the beginning of the titration, the CD spectrum resembles that of poly[d(A)]·poly[d(T)], as can be expected on the basis of the sequence.[24] The spectra recorded upon the addition of Ag^+ show a trend similar to that observed for a titration of poly(A)·poly(U) with Ag^+.[25] In the latter case, this change had been attributed to the binding of the metal ion to all of the nucleoside residues and subsequent alteration of the electronic configuration of the bases and their interactions within the polynucleotide.[25] At first glance, these results are in contradiction to those from the melting experiments, which suggested specific binding of the silver(I) ions. UV spectra of d($A_7X_3T_7$) with various amounts of $AgNO_3$ present in the solution, recorded concomitantly with the CD spectra, help to shed light on this apparent contradiction. As can be seen

(21) One equiv of metal ions represents the amount that is necessary for the formation of a regular double helix in which each potential metal-ion-mediated base pair contains one metal ion.

(22) The labeled oligonucleotides **A**, **B**, and **C** cannot be used to confirm the conformational change. In these, the fluorescein and dabcyl moieties stabilize the hairpin conformation to such an extent, that no regular double helix is formed upon the addition of silver(I). This can be seen for example from the concentration-independent melting temperature of **C** (Supporting Information, Figure S2).

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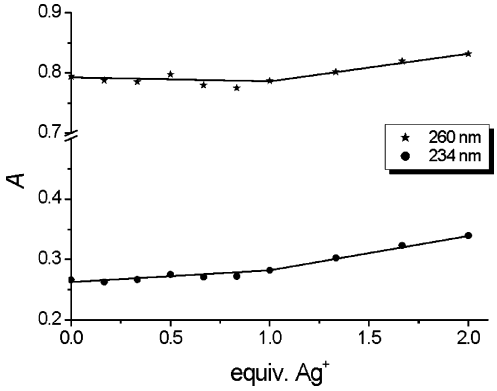

Figure 5. Changes in the UV spectrum of d($A_7X_3T_7$) upon the addition of $AgNO_3$. The abrupt change in the slope at 1 equiv of Ag^+ clearly indicates that the first equiv interacts differently with the oligonucleotide than the subsequent ones. Conditions: 6.4 μM d($A_7X_3T_7$), 150 mM $NaClO_4$, 5 mM MOPS pH 6.8.

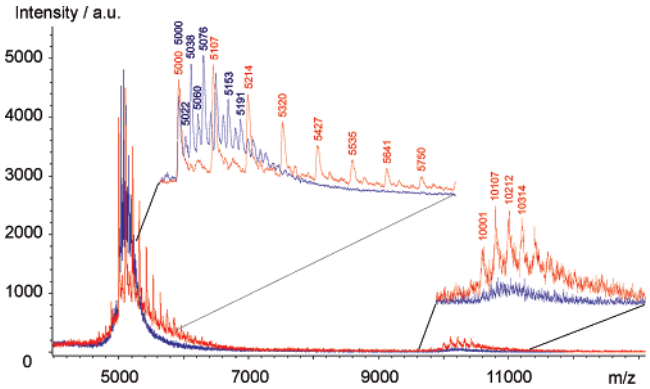

Figure 6. Sections of MALDI-TOF spectra of d($A_7X_3T_7$) in the absence (blue) and presence of 5 equiv of Ag^+ (red). Selected calculated values for m/z ($z = +1$) are as follows: single strand, 5002; single strand + Ag^+: 5109; single strand + 2 Ag^+: 5216; single strand + 3 Ag^+: 5323; double helix: 10 003; double helix + Ag^+: 10 110; double helix + 2 Ag^+: 10 217; double helix + 3 Ag^+: 10 323. In addition, Na^+ and K^+ adducts are observed (+ 22 and + 38, respectively, with regard to the original peak).

from Figure 5, no changes are observed in the UV spectrum upon the addition of the first equivalent of silver(I), whereas additional silver(I) leads to significant changes.

This clearly shows that the first equiv of Ag^+ interacts differently with the oligonucleotide than excess Ag^+, going along well with the preferential formation of three metal-ion-mediated triazole base pairs prior to a different interaction of the excess silver(I). As triazole nucleoside does not have any significant absorbance above 220 nm (Supporting Information, Figure S4), no changes are expected in that spectral region upon the formation of metal-ion-mediated triazole base pairs.

The results of MALDI-TOF mass spectrometric studies can also be explained in terms of a silver(I)-induced stabilization of the double helix, with the most likely explanation being the formation of metal-ion-mediated base pairs. The mass spectrum of d($A_7X_3T_7$) in the presence of 5 equiv of $AgNO_3$ indicates that most of the double helix dissociates under the experimental conditions, as it shows peaks corresponding to the single strand (calculated for $(M_1+H)^+$: m/z = 5002 Da) with distinct silver(I) adducts (Figure 6, red spectrum). However, the same spectrum shows

Table 2. Results of the DLS Measurements[a]

oligonucleotide	r (nm)	mass (%)	% polydispersity
d($A_7X_3T_7$)	1.60 ± 0.10	100	7.3
d($A_7X_3T_7$) + Ag^+	2.10 ± 0.05	99.9	7.4
reference hairpin	1.55 ± 0.05	99.9	8.4
reference double helix	2.05 ± 0.05	99.9	7.0

[a] The hydrodynamic radius given in this table corresponds to the average value from at least four measurements. Reference hairpin: d(A_7GAAT_7); reference double helix: d(GGAAAAGGAGAGAAGAA)·d(TTCTTCTC-TCCTTTTCC).

− albeit at lower intensity − peaks that can be assigned to the respective regular double helix (calculated for $(M_2+H)^+$: m/z = 10 003 Da), again including silver(I) adducts. Although MALDI-TOF measurements are known for their tendency to produce casual binary adducts such as $(2M_1+H)^+$ that cannot be distinguished from specifically formed binary adducts like the double helix $(M_2+H)^+$, such a behavior is unlikely in the case discussed here, as can be seen from a comparison with the MALDI-TOF spectrum of the same sample, prior to the addition of silver(I) (Figure 6, blue spectrum). Under these conditions, only the hairpin structure is formed, hence any peak around m/z = 10 000 can only be due to a casual adduct. Indeed, the spectrum, displaying various Na^+ and K^+ adducts for the single strand, shows a much smaller peak corresponding to the binary adduct. Hence, the increase in intensity of the peaks around m/z = 10 000 in the presence of silver(I) clearly shows that the addition of these metal ions induces double-helix formation. The MALDI-TOF spectra of a different batch of d($A_7X_3T_7$) illustrate this trend in an even more pronounced fashion, albeit at a lower spectral resolution and with more alkali metal-ion adducts (Supporting Information, Figure S5).

Final proof for the conformational change from hairpin to regular double helix comes from dynamic light scattering (DLS) experiments. Table 2 shows the results of these measurements.

In the absence of any transition-metal ions, when d($A_7X_3T_7$) adopts a hairpin conformation, a solution of d($A_7X_3T_7$) comprises 100 mass% of a particle with a mean hydrodynamic radius of 1.60 ± 0.10 nm. This value lies in the expected range for a hairpin structure, considering an average helical rise of 0.34 nm between individual base pairs and a considerable contribution of the loop to the overall size of the particle. The addition of $AgNO_3$ to the solution leads to a mass distribution with 99.9 mass% belonging to a particle with a mean hydrodynamic radius of 2.10 ± 0.05 nm. This increase in size is expected when going from a hairpin with 7 base pairs to a regular double helix comprising 17 base pairs, as can be seen from the concomitantly performed reference measurements (Table 2). In the latter, oligonucleotides that do not contain artificial nucleobases and that form a hairpin and a double helix, respectively, show mean hydrodynamic radii of 1.55 ± 0.05 (hairpin) and 2.05 ± 0.05 nm (double helix). Within the error limits, these values are in excellent agreement with those of the nucleic acids

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containing triazole nucleoside, hence confirming the conformational change from hairpin to double helix upon the addition of silver(I).[26]

Conclusions

We have shown that artificial nucleosides can be used to synthesize oligonucleotides that are capable of undergoing a conformational change upon the addition of appropriate transition-metal ions. More precisely, the oligonucleotide sequence $d(A_7X_3T_7)$ (with X = 1,2,4-triazole) adopts a hairpin structure in which the artificial nucleosides are located in the loop. As a result of their lacking capability to engage in hydrogen bonding, the corresponding regular double helix that would contain three X···X mispairs is destabilized. However, in the presence of silver(I) ions, this very double helix is formed preferentially, because now the triazole nucleosides can form metal-ion-mediated base pairs. This concept of modifying the nucleic acid conformation by the addition of metal ions provides the opportunity to generate a large variety of metal-ion sensors, with the possibility of fine-tuning their sensing capabilities depending on the artificial nucleoside that is used, or on the sequence of the hairpin stem that is built from regular nucleosides. Work is in progress in our laboratory to exploit this conformational change in terms of a fluorescent turn-on sensor.

Acknowledgment. Financial support from the Deutsche Forschungsgemeinschaft (Emmy Noether Program), the Fonds der Chemischen Industrie, and the Department of Chemistry at the University of Dortmund is gratefully acknowledged. J.M. thanks Prof. Dr. Bernhard Lippert for his continuous support. We thank Silke Johannsen, Dr. Eva Freisinger, and Prof. Dr. Roland K. O. Sigel (University of Zurich, Switzerland) for access to the DLS instrument and for performing some of the measurements as well as Prof. Dr. Christof M. Niemeyer (University of Dortmund) for access to the fluorescence spectrophotometer.

Supporting Information Available: Melting curves of $d(A_7X_3T_7)$ in the presence of Hg^{2+}, melting temperatures of **C** in dependence of Ag^+ and oligonucleotide concentrations, CD spectra of $d(A_7X_3T_7)$ in the presence of Ag^+, UV spectrum of the 1,2,4-triazole nucleoside, MALDI-TOF spectra of $d(A_7X_3T_7)$ in the presence of various equivalents of Ag^+.This material is available free of charge via the Internet at http://pubs.acs.org.

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(26) The hydrodynamic radius does not double when the length of the oligonucleotide doubles because it merely represents the radius of a *hypothetic* hard spherical particle that diffuses with the same speed as the particle under examination. Whereas short hairpins can accurately be described as spherical particles, double helices should rather be considered as short, rodlike molecules.[27] Hence, the hydrodynamic radii of the latter are always smaller than half of the length of their long axes.

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