DNA Structures

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Controlled Stacking of 10 Transition-Metal Ions inside a DNA Duplex**

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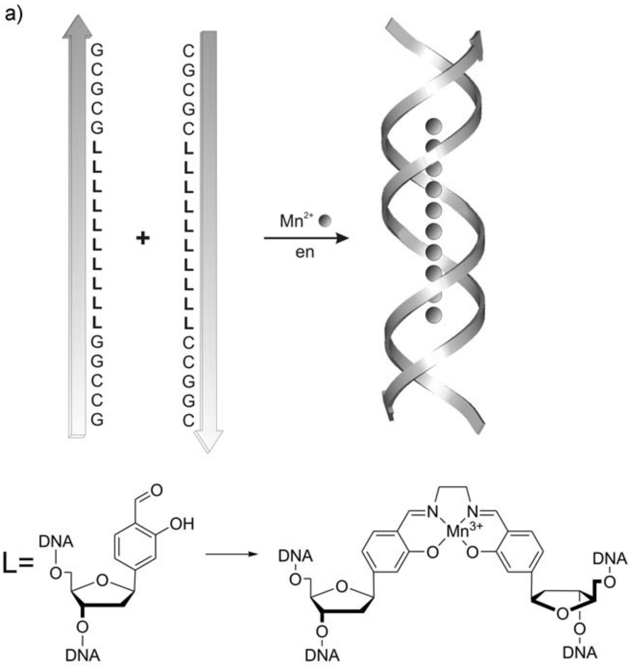
The controlled assembly of functional nanoscale materials from molecular entities is a key issue for future nanotechnological developments. Currently, the use of DNA, which has superior self-organization properties, in such assembly processes is under intense investigation.[1] Recently, the assembly of DNA-based nanostructures of fascinating complexity has been reported.[2] One current goal is the development of methods to impart these DNA constructs with specific functions, such as electrical conductivity or single-molecule magnetism.

A recent advance is the replacement of Watson–Crick base pairs by metal–base pairs, in which the hydrogen bonds that determine the selective pairing of the nucleobases are replaced by coordination interactions between ligands and metal ions.[3] Several groups have reported the incorporation of more than one metal ion into a single DNA double strand; however, these reports have mainly been based on melting-point experiments.[4] Studies on the targeted stacking of Hg^{2+} ions inside DNA duplexes are particularly noteworthy.[5,6] However, the unspecific binding of Hg^{2+} ions to the natural DNA bases, a well-known phenomenon,[7] was a problem in some cases.[5] Shionoya and co-workers recently synthesized and characterized a DNA system allowing the complexation of up to five Cu^{2+} ions inside a double helix.[8]

We recently described a metal–salen base pair in which a variety of metal ions can serve as coordination partners to the *N*,*N'*-bis(salicylidene)ethylenediamine (salen) ligand.[9] An advantage of this concept is that the ethylenediamine (en) used to assemble the metal–salen complexes inside the DNA double strand also forms a covalent crosslink, imparting an unusually high stability to the duplex. In fact, the resulting duplex stabilization is far higher than that observed for any other metal–base pair.[9,10] We reasoned that this unusual stabilization could allow the construction of stable DNA structures containing more than one transition-metal ion stacked on top of one another. This approach could provide access to programmable DNA-based multinuclear coordination complexes.

The underlying assembly process of the metal–salen base pairs is shown in Figure 1 a. Two complementary DNA single strands, each containing multiple salicylic aldehyde (**L**) nucleobases, are hybridized into a double strand. A subsequent reaction with ethylenediamine links pairs of opposite salicylic aldehyde nucleobases, forming salen ligands. Upon addition of Cu^{2+} or Mn^{2+} ions, a specific number of metal ions are complexed by the salen ligands. The largest hurdle to be overcome in the stacking of a large number of metal ions inside a DNA double strand is an uncontrolled slippage of the single strands relative to one another (frameshift), which would result in the formation of mismatched metal–base pairs and unoccupied ligand sites.

We prepared duplex **1-L_2-a/b**, which contains precursor bases for two isolated salen ligands separated by three



b)

1-L_2-a: 5'-CGGA**L**GAC**L**AGCG-3'
1-L_2-b: 3'-GCCT**L**CTG**L**TCGC-5'

2-L_2-a: 5'-CACATT**LL**TGTTGTA-3'
2-L_2-b: 3'-GTGTAA**LL**ACAACAT-5'

3-L_5-a: 5'-GCGCG**LLLLL**GGCCG-3'
3-L_5-b: 3'-CGCGC**LLLLL**CCGGC-5'

4-L_{10}-a: 5'-GCGCG**LLLLLLLLLL**GGCCG-3'
4-L_{10}-b: 3'-CGCGC**LLLLLLLLLL**CCGGC-5'

Figure 1. a) The assembly of 10 metal–salen base pairs inside a DNA duplex. b) The modified oligonucleotides prepared for this study. **L** represents the salicylic aldehyde nucleobases, which serve as precursors for the formation of metal–salen complexes.

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Watson–Crick base pairs, and duplex **2-L_2-a/b**, which contains precursor bases for two adjacent salen ligands (Figure 1b). To investigate the complexation behavior of each duplex, we mixed the two complementary single strands, and then added ethylenediamine and the metal salt. An excess of ethylenediamine was always used, whereas a stoichiometric amount of the metal salt was added.

The reaction mixtures were characterized by electrospray ionization (ESI) ion cyclotron resonance (ICR) mass spectrometry, which yielded signals corresponding to the expected products [**1-L_2-a/b** $+ 2\,en + 2\,Cu^{2+} - 4\,H_2O - 4\,H^+$] and [**2-$L_2$-a/b** $+ 2\,en + 2\,Cu^{2+} - 4\,H_2O - 4H^+$]. No signals due to uncomplexed single strands were observed in the mass spectra. Both duplexes clearly contain two copper–salen complexes (see Supporting Information).

Furthermore, a melting-point study of duplex **1-L_2-a/b** in the absence and presence of ethylenediamine and two equivalents of Cu^{2+} ions yielded curves consistent with our proposed assembly scheme (Figure 2). In the absence of ethylenediamine or metal ions, **1-L_2-a/b** melts at $T_M = 34.2\,°C$. The addition of ethylenediamine shifted the melting temperature to a higher value and produced a characteristic hysteresis (average $T_M = 41.6\,°C$). This hysteresis can be explained on the basis of the reversibility of the imine linkage between the salicylic aldehydes and ethylenediamine.[10] When one equivalent of Cu^{2+} ions was added, the system showed two sigmoidal transitions. The observation of two melting transitions, one at $T_M = 41.2\,°C$ (with hysteresis) and a second at $T_M = 82.9\,°C$ (without hysteresis) indicates the presence of two well-defined species in solution. We assume that, under the conditions of the thermal de- and renaturing experiment, the assembly of duplexes containing two Cu^{2+} ions proceeds cooperatively. When only one equivalent of Cu^{2+} ions is added, half of the duplexes are without metal ions ($T_M = 41.6\,°C$), and the other half contain two metal ions ($T_M = 82.9\,°C$). This hypothesis is also supported by the detection of a signal corresponding to a duplex containing two Cu^{2+} ions, [**1-L_2-a/b** $+ 2\,en + 2\,Cu$], in the mass spectrum of this sample. The addition of a second equivalent of Cu^{2+} ions resulted in only one well-defined melting point at $T_M \approx 92.1\,°C$, which is indicative of the exclusive formation of DNA duplex species containing two Cu^{2+} ions, [**1-L_2-a/b** $+ 2\,en + 2\,Cu$]. An analogous melting-point study with duplex **2-L_2-a/b** also yielded melting profiles that underwent significant changes upon addition of one or two equivalents of Cu^{2+} ions; however, the curves in this case were more complex. The results of the melting-point and mass spectrometric studies are consistent with the formation of DNA duplexes that contain two metal–salen complexes.

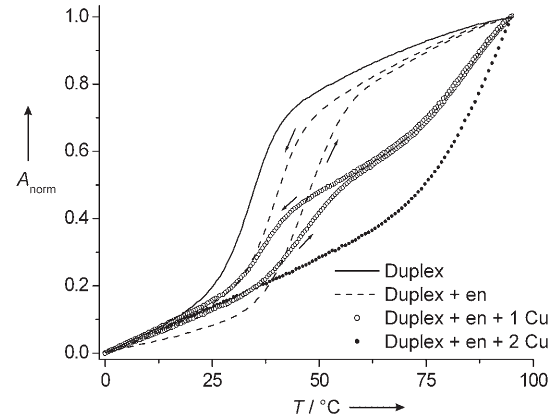

Figure 2. Comparison of the melting curves recorded for duplex **1-L_2-a/b** without additives (——), with excess ethylenediamine (-----), with ethylenediamine and one equivalent of Cu^{2+} ions (○), and with ethylenediamine and two equivalents of Cu^{2+} ions (●). The samples contained 3 μM duplex, 10 mM *N*-cyclohexyl-2-aminoethanesulfonic acid (CHES) buffer, and 150 mM NaCl.

To expand the system to the complexation of 5 or 10 transition-metal ions, we synthesized the oligonucleotides **3-L_5-a/b** and **4-L_{10}-a/b**, according to reported protocols.[10] The sequences of five Watson–Crick base pairs on the two ends of duplexes **3-L_5-a/b** and **4-L_{10}-a/b** were chosen to optimize the pre-organization of the double strands for subsequent complex formation.

The characteristic changes in the UV/Vis spectrum of duplex [**4-L_{10}-a/b** $+ 10\,en$] that occur upon titration with Cu^{2+} ions are shown in Figure 3a. The overlaid curves exhibit isosbestic points at $\lambda = 342$ and 398 nm. A plot of the intensity of the absorption maximum of the copper–salen chromophore at $\lambda = 360$ nm against the equivalents of Cu^{2+} ions

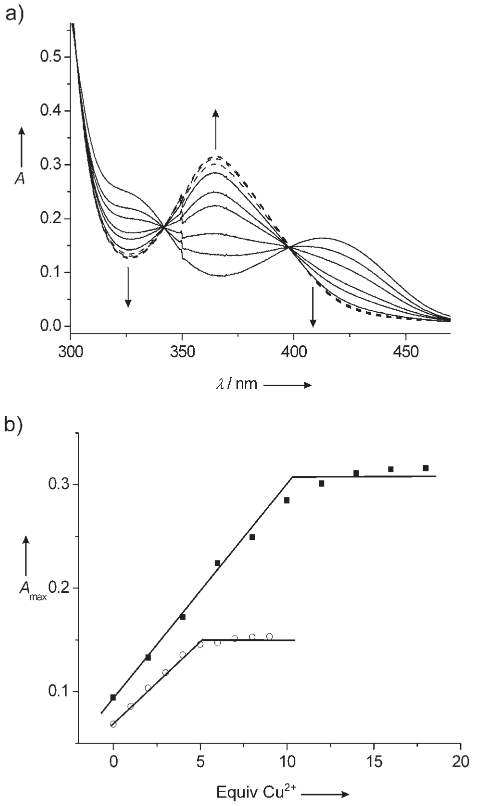

Figure 3. Titration of [**3-L_5-a/b** $+ 5\,en$] and [**4-L_{10}-a/b** $+ 10\,en$] with Cu^{2+} ions. a) Overlay of UV/Vis spectra of [**4-L_{10}-a/b** $+ 10\,en + x\,Cu$] (6 μM) at various concentrations of Cu^{2+} ions (from 0 to 108 μM in steps of 12 μM); b) Plot of the intensity of the absorption maximum against the equivalents of Cu^{2+} ions for [**3-L_5-a/b** $+ 5\,en + x\,Cu$] (○) and [**4-L_{10}-a/b** $+ 10\,en + x\,Cu$] (■; see Supporting Information). The samples contained 10 mM CHES buffer and 150 mM NaCl.

increases linearly until a duplex/Cu^{2+} ratio of approximately 1:10 is reached (Figure 3b). Analogous titration curves consistent with a duplex/Cu^{2+} ratio of 1:5 were obtained for [**3-L$_5$-a/b** + 5en + 5Cu] (Figure 3b and Supporting Information).

The circular dichroism (CD) spectra recorded at 0–80 °C for duplex **4-L$_{10}$-a/b** before and after the assembly of the 10 manganese–salen complexes are depicted in Figure 4.

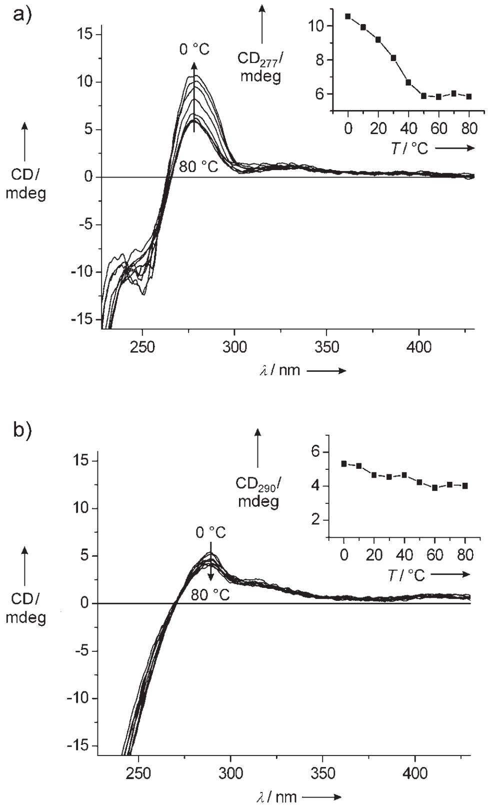

Figure 4. CD spectra of duplex **4-L$_{10}$-a/b** at 0–80 °C in steps of 10 °C. a) 3 μM duplex, 10 mM *N*-(2-hydroxyethyl)piperazine-*N'*-2-ethanesulfonic acid (HEPES) buffer, 150 mM NaCl (80→0 °C). b) The same sample after incubation for 24 h with 1 mM ethylenediamine and 40 μM Mn^{2+} ions (0→80 °C). Insets: plots of the magnitude of the positive CD maximum against temperature. Blank measurements were subtracted at each temperature.

Prior to the addition of ethylenediamine and Mn^{2+} ions, the spectra featured a positive band near $\lambda = 277$ nm, a negative band (shoulder) near $\lambda = 249$ nm, and a zero crossing at $\lambda = 265$ nm. These features imply that the duplex has a secondary structure similar to that of B-DNA at room temperature.[11] When the sample was slowly cooled from 80 to 0 °C, the CD spectra changed significantly (Figure 4a). The magnitude of the positive CD maximum is plotted against temperature in the inset to Figure 4a. This curve suggests that **4-L$_{10}$-a/b** melts near 35 °C, in accordance with corresponding melting-point curves determined by UV/Vis spectroscopy (not shown).

Upon addition of excess ethylenediamine and Mn^{2+} ions, the CD spectrum changed significantly: the magnitude of the positive band near $\lambda = 290$ nm decreased, and the band near $\lambda = 249$ nm vanished completely (Figure 4b). A clear classification of the secondary structure of [**4-L$_{10}$-a/b** + 10en + 10Mn] on the basis of this data is difficult. An investigation of the temperature dependence of the spectra, however, led to an important observation: the spectrum of [**4-L$_{10}$-a/b** + 10en + 10Mn] remained practically unchanged at temperatures between 0 and 80 °C (Figure 4b, inset). This behavior is a clear indication of the high structural stability of the duplex expected to result from the multiple metal–salen crosslinks.

To obtain further support for the assembly of 5 and 10 metal–salen complexes inside double strands **3-L$_5$-a/b** and **4-L$_{10}$-a/b**, ESI-ICR mass spectrometric experiments were performed. Owing to the sensitivity of the technique towards even weakly associated adducts, unspecific binding of the metal ions to the DNA duplex had to be avoided. In the case of the Cu^{2+}-ion complexation, strict control of the DNA concentration and the amount of Cu^{2+} ions added was necessary. The use of Mn^{2+} ions, which showed less tendency to form unspecific complexes with the DNA duplexes, produced cleaner mass spectra.

In the case of [**3-L$_5$-a/b** + 5en], the addition of exactly five equivalents of Cu^{2+} ions yielded a mass spectrum containing the signals expected for [**3-L$_5$-a/b** + 5en + 5Cu]; however, the addition of 10 equivalents of Cu^{2+} ions yielded a mass spectrum that could be assigned to the species [**3-L$_5$-a/b** + 5en + 9Cu] (see Supporting Information). The use of Mn^{2+} instead of Cu^{2+} ions changed this picture: according to the mass spectra, the desired number of metal ions were complexed by the double strands under all circumstances. The addition of ethylenediamine and Mn^{2+} ions to duplex [**4-L$_{10}$-a/b**] yielded a mass spectrum corresponding to a structure containing exactly 10 manganese–salen complexes. The results of these ESI-ICR mass spectrometric experiments are shown in Figure 5. The expected signals for the product [**4-L$_{10}$-a/b** + 10en + 10Mn^{3+}−20H$_2$O−20H$^+$]$^{10+}$ are clearly

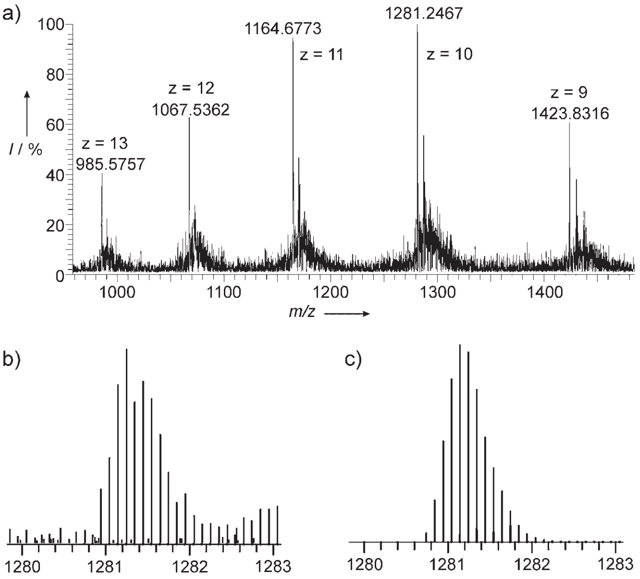

Figure 5. a) The ESI-ICR mass spectrum of [**4-L$_{10}$-a/b** + 10en + 10Mn]. The *m/z* values for $z = 9$–13 are in excellent agreement with the calculated masses. b) Measured isotope pattern for [**4-L$_{10}$-a/b** + 10en + 10Mn^{3+}−20H$_2$O−40H$^+$]$^{10-}$. c) Simulated isotope pattern for $[C_{450}H_{502}N_{100}O_{236}P_{38}Mn_{10}]^{10-}$.

visible. Thus, duplex **4-L$_{10}$-a/b** reacted with 10 molecules of ethylenediamine and 10 manganese ions with the loss of 20 equivalents of water. Salen-bound Mn^{2+} ions are known to be oxidized to Mn^{3+} ions under aerobic conditions.[12] The mass spectrum is in agreement with this assumption: the complex [**4-L$_{10}$-a/b** + 10 en + 10 Mn] contains 10 Mn^{3+} ions. The metal coordination sites that are not occupied by the tetradentate salen ligand are probably saturated by loosely bound water (or acetate) molecules or by interactions with the donor atoms of a neighboring salen ligand or nucleobase.

In summary, we have shown that DNA duplexes containing 20 salicylic aldehyde "nucleobases" react with ethylenediamine to form 10 stacked salen ligands inside the double helix. These ligands can subsequently coordinate 10 transition-metal ions, such as Cu^{2+} or Mn^{2+}. The reversibility of the formation of the salen ligands and the complexation steps allows the system to reach thermodynamic equilibrium. This hypothesis could explain why no frameshift, which would give rise to incomplete complexation, occurs. Up to 10 metal ions can be stacked inside a DNA duplex, representing a full helical turn with respect to the structure of native B-DNA. The stack of 10 metal–salen complexes inside a double helix of [**4-L$_{10}$-a/b** + 10 en + 10 Mn] is depicted in Figure 6.

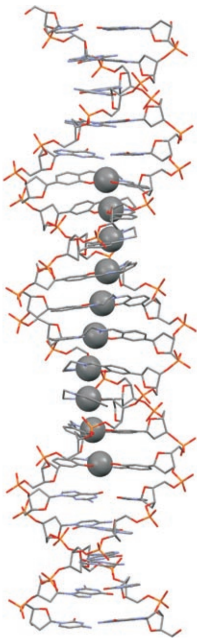

Figure 6. The computer-generated model of [**4-L$_{10}$-a/b** + 10 en + 10 Mn] represents a plausible arrangement of the 10 metal–salen complexes inside the DNA double helix. C gray, N blue, O red, P orange, Mn gray spheres; H omitted.[14]

We believe that a combination of the metal–base pair concept with the well-established sequence-based techniques for the construction of complex DNA architectures with branches and junctions[13] will soon allow the synthesis of programmable constructs containing several metal binding sites in specific positions, leading to interesting prospects for molecular electronics and the mimicry of multimetal enzymes.

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[14] Manual fitting of the crystal structure of the copper–salen complex (determined by X-ray diffraction) into an idealized model of a double strand of B-DNA with a nucleobase separation of 3.375 Å and a twist of 36° per base (Spartan '02, Wavefunction Inc., Irvine (USA), **1991–2002**).