DNA Nanomaterials

DOI: 10.1002/anie.200906359

Antiferromagnetic Coupling of Stacked Cu^{II}–Salen Complexes in DNA**

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On the way to novel bottom-up generated functional materials that might play a key role in future nanotechnology, the use of DNA has become a major strategy.[1] The relative ease of automated DNA synthesis has been exploited to build a wide range of two- and three-dimensional structures from unmodified[2] and modified DNA strands.[3] Currently, the implementation of real functions such as long-distance electron transfer or molecular magnetism is pursued as the next major step. As a promising candidate for the realization of such functions, the concept of metal–base pairing has been developed.[4] In metal–base pairs, the natural hydrogen-bonding interactions between the complementary nucleobases are substituted by coordinative forces between ligand-modified nucleosides and appropriate transition-metal ions. It has been shown that stacks of up to 10 metal ions such as Cu^{II} can be incorporated inside DNA double helices that are modified with ligands such as hydroxypyridone (**H**) or *N*,*N*′-bis(salicylidene)ethylene diamine (salen, **S**).[5] Apart from the superior duplex stabilization that was achieved using these systems, the positioning of a number of paramagnetic ions inside these DNA materials was envisioned to yield DNA strands with ferro- or antiferromagnetic behavior. The basis for this behavior is the electron–electron spin–spin exchange coupling *J* between the Cu^{II} ions.[6] An understanding of structural correlations such as intermetallic distance and base sequence giving rise to the sign and size of *J* is a prerequisite for the rational design of magnetic DNA strands. A first step in this direction was the observation of an overall ferromagnetic coupling in a stack of five Cu^{II}–hydroxypyridone [$\mathbf{H}_2$(Cu)] metal–base pairs by Shionoya and co-workers.[7]

Herein, we introduce electron paramagnetic resonance (EPR) based magnetic measurements performed on the Cu^{II}–salen metal–base pairs [**S**(Cu)] inside the DNA double helix (Figure 1).[8] Interestingly, changing the ligand system from hydroxypyridone to salen induces a profound change to an overall antiferromagnetic coupling. In the $\mathbf{H}_2$(Cu) metal–base pair, two separate bidentate hydroxypyridone ligands coordinate to the Cu^{II} ion through four oxygen atoms. In contrast, the salen ligand **S** is a tetradentate N_2O_2 donor, which is realized as a cross-link between the two strands of the DNA duplex.[9] Although in both systems, Cu^{II} is coordinated in a square-planar fashion inside the DNA double helix, subtle differences of the coordinating ligands seem to effect the magnetic interaction of neighboring metal–base pairs in a tremendous way.

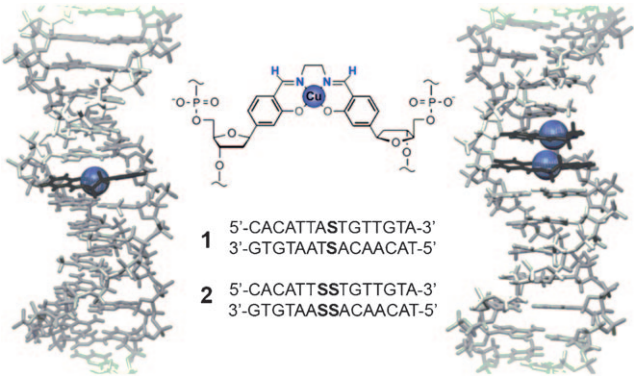

Figure 1. Structure of the **S**(Cu) metal–base pair (nuclei giving rise to hyperfine splitting highlighted in blue), sequences of the examined duplexes containing one (**1**) and two (**2**) Cu^{II} ions, and schematic depiction of the envisioned structures of the metal containing double strands.

As a reference system and to check whether the Cu^{II}–salen structure is preserved in DNA, low-temperature continuous-wave (CW) X-band EPR spectra were acquired from DNA **1** containing a single Cu^{II}–salen base pair. A representative EPR spectrum of **1** is displayed in Figure 2a and shows the characteristic features of a square-planar Cu^{II} system with $S = {}^1/_2$. Simulating the spectrum yields the spin Hamiltonian parameters summarized in Table 1. The hyperfine and super hyperfine features are well reproduced and consistent with the expected interactions of the Cu^{II} ion with two equivalent nitrogen atoms with nuclear spins $I(^{14}N) = 1$ and two equivalent and weakly coupled hydrogen atoms with nuclear spins of $I(^1H) = {}^1/_2$ (exemplified in Figure 1). The axial coordination symmetry and the EPR parameters fit to those reported for reported pure inorganic Cu^{II}–salen structures.[10]

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[**] This work was supported by the BBSRC (BB/F004583/1), the Volkswagen-Stiftung, and the SFB 749. G.H.C. and S.J.R. thank the Studienstiftung des Deutschen Volkes and the Stiftung Stipendien-Fonds des Verbands der Chemischen Industrie e.V. for fellowships.

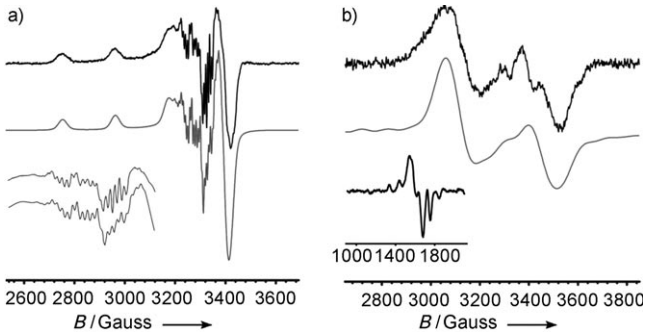

Figure 2. CW X-band EPR spectra of a) **1** and b) **2** at 77 K (black) overlaid with the simulations (gray). The inset in (a) shows an enlargement of the perpendicular region and the inset in (b) shows the half-field signal recorded at 14 K.

Table 1: EPR parameters for a monomeric Cu^{II}–salen complex **S**(Cu) from the literature[10] and for DNA duplexes **1** and **2**.

	S(Cu)	DNA **1**	DNA **2**
g_{iso}	2.094	2.093	2.098
$g_{\parallel}$	2.194	2.194	2.194
$g_{\perp}$	2.041	2.042	2.054
$A_{iso}(Cu^{2+})$ [G]	90.4	91.4	91.9
$A_{\parallel;\perp}(Cu^{2+})$ [G]	221.8; 31.8	208.5; 32.8	210.0; 32.8
$A(^{14}N)$ [G]	15.5	16.0	16.0
$A(^{1}H)$ [G]	7.1	7.5	7.5
D [G]	–	–	370 ± 10
$J_{average}$ [cm^{-1}]	–	–	-11.2 ± 1

This result confirms that upon self-assembled duplex formation, the geometry of the inorganic complex is preserved.

In DNA duplex **2**, two Cu^{II}–salen base pairs are direct neighbors of each other, which induces strong coupling between the two $S = {}^1/_2$ systems, leading to the formation of a coupled spin state with a singlet ($S = 0$) and a triplet ($S = 1$) state. At a magnetic field B of around 3300 G, DNA duplex **2** shows a broad signal due to $\Delta m_s = \pm 1$ transitions between the $m_s = 0$ and $m_s = \pm 1$ levels within the triplet state. The shape of this signal is dominated by the dipolar through space interaction D between the two Cu^{2+} centers (Figure 2b).[11] Simulating this spectrum yields the EPR parameters in Table 1, the hyperfine and g tensors of which are very similar to those of DNA **1**. A dipolar coupling constant D of 370 ± 10 G was obtained for the two Cu^{II} ions. A distance r of 3.7 ± 0.1 Å was calculated from this value by utilizing Equation (1), which is based on the point dipole approximation.[11] It is known that the point dipole approximation may break down at such short distances, for example, because of spin delocalization or anisotropic exchange coupling contributions.[12] A contribution of the latter cannot be excluded, but the effect of spin delocalization is expected to be minor because the two metal–base pairs are stacked and not aligned in a side-to-side fashion.

$$r = \sqrt[3]{\frac{18.6\,[\mathrm{G}]}{D\,[\mathrm{G}]}}\,[\mathrm{nm}] \qquad (1)$$

The coupling between the two Cu^{II} centers also gives rise to the so-called half-field signal centered at around $B = 1600$ G, which is due to the forbidden $\Delta m_s = \pm 2$ transition between the $m_s = +1$ and $m_s = -1$ levels within the triplet state. Since this signal can only originate from DNA strands with two coupled copper ions, its intensity is not compromised by monoradical impurities. With the exchange coupling constant J being defined as the energy separation between the singlet and triplet state and with the singlet state being EPR-silent, the temperature-dependent intensity of this half-field signal provides a means of measuring the value of J. The inset in Figure 2b shows this seven line signal with a line splitting of 95 G, which is approximately half of the $A_{\parallel}(Cu^{2+})$ hyperfine coupling. Plotting the intensity I of this signal against the temperature T at which it was recorded enables one to extract the sign and magnitude of J by fitting the resulting curve to a Bleany–Bowers-type Equation (2), where k and C are the Boltzmann constant and a spectrometer constant obtained from the fit as $9.6 \times 10^{-6}\,cm^{-1}$, respectively.[13]

$$I(T) = \frac{C}{T}\left(\frac{1}{3 + \exp(-2J/kT)}\right) \qquad (2)$$

In Figure 3, the half-field signal intensity measured as peak-to-peak height of the predominant peak is plotted against T for DNA duplex **2**. Best fits to Eq. (**2**) reveal an

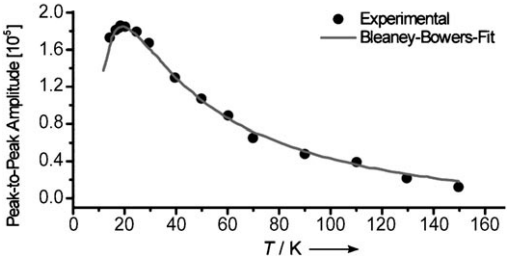

Figure 3. Intensity of the half-field signal of DNA **2** plotted versus temperature T (circles) and best fit to equation (**2**) (solid line).

exchange coupling constant of $J = -10.8 \pm 0.2\,cm^{-1}$. Using a plot of the doubly integrated intensity against T results in a similar value of $J = -11.6 \pm 0.2\,cm^{-1}$ (data not shown, $J_{average} = -11.2 \pm 1\,cm^{-1}$). Thus, stacking of two Cu^{II}–salen base pairs leads to antiferromagnetic coupling, whereas ferromagnetic coupling was reported for stacked Cu^{II}–hydroxypyridone base pairs.[7] Such a change in magnetism upon switching from Cu^{II}–hydroxypyridone to Cu^{II}–salen base pairs was predicted recently in two independent DFT studies by Nakanishi et al.[14] and by Mallajosyula and Pati.[15] In the latter work, an exchange coupling constant of $J = -10.5\,cm^{-1}$ was calculated for the Cu^{II}–salen system based on a predicted square-planar geometry and a Cu···Cu distance of 3.7 Å. The magnitude and sign of J as well as the geometry agree very well with the EPR data reported herein, which renders also the suggested mechanisms as very likely. The pairwise arrangement and the rather long Cu···Cu distance in the

S(Cu)–DNA system leads to a weaker and antiferromagnetic coupling due to a direct cation–cation exchange-coupling mechanism, which is in agreement with the Goodenough–Kanamori rules.[15] In contrast, the so-called four-atom $\{Cu_2O_2\}$ convex quadrangle structure in the $\mathbf{H_2}$(Cu) system arranges the magnetic orbitals in an orthogonal fashion, which suppresses the antiferromagnetic interaction and together with the shorter Cu···Cu distance of 3.2 Å leads to a strong ferromagnetic interaction. It seems that the oxygen atom contacts with the neighboring bases to form the $\{Cu_2O_2\}$ quadrangle, which provides a bridge for exchange pathways in the $\mathbf{H_2}$(Cu) system, whereas such contacts could not be resolved in the **S**(Cu) system.[15] An additional ligand such as water bridging the two copper ions in either of the two systems, [$\mathbf{H_2}$(Cu)] or [**S**(Cu)], could not be verified either by high-resolution ESI mass spectra or from the EPR spectra. This result shows that the incorporation of stacked Cu^{II}–base pairs into DNA does not automatically result in ferromagnetic coupling and precise experimental and theoretical studies are required.

In summary, we characterized the magnetic properties of novel metal–DNA species containing one and two Cu^{II} ions. The data show that the axial, square-planar geometry of the original inorganic complex is preserved inside the DNA duplex. Furthermore, the exchange coupling between the two paramagnetic metal–base pairs inside the DNA strand was quantified by temperature-dependent CW EPR measurements and yielded an antiferromagnetic exchange coupling constant of $J_{average} = -11.2 \pm 1.0\ cm^{-1}$. The experimental data provided herein for the **S**(Cu) DNA and the data reported for the $\mathbf{H_2}$(Cu) system are in good agreement with the respective theoretical predictions, thus providing the basis for future investigations of the magnetic properties of artificial metal–DNA complexes. Because of the electron-transfer properties of metal–DNA complexes and the possibility to generate mixed-metal arrays[16] in a sequence-specific manner, the present findings are a further step towards the rational construction of devices in the emerging field of molecular spintronics.[17]

Experimental Section

The synthesis and characterization of the DNA duplexes **1** and **2** have been described previously.[8] Careful addition of stoichiometric amounts of Cu^{2+} to a solution of the hybridized DNA duplexes resulted in the exclusive formation of the desired DNA–metal complexes as monitored by ESI-FTICR mass spectrometry and UV spectroscopy. The CW X-band EPR spectra were acquired between 13 K and 150 K in frozen solution on a BRUKER ESP300E spectrometer with a standard rectangular ER4102T cavity equipped with an Oxford Instruments helium cryostat (ESR910/900). In Figure 3, data points below 10 K were omitted because of the strong decrease in signal intensity and a concomitant significant increase in spectral noise below this temperature. Solutions of the DNA–metal complexes (360 μM) in NH_4OAc buffer (100 mM) at pH 8 containing ethylenediamine (5 mM) were used. Ethylene glycol (20% v/v) was added to the aqueous buffer as a cryoprotectant and all samples were stored instantaneously under liquid nitrogen. Simulations of the spectra were preformed with WINEPR SimFonia 1.25 and EasySpin 2.51.[18] In the simulations of DNA **1**, linewidths of 7 G for the x and y and 32 G for the z directions were used. The ratio between Lorentzian and Gaussian line shapes was set to 0.3. For the simulations of the EPR spectrum of DNA **2**, a spin-Hamiltonian with two interacting $S = {}^1/_2$ centers including the terms SDS and JSS was used. The line widths were 80 G for x and y and 65 G for the z directions.

Received: November 11, 2009
Revised: March 20, 2010
Published online: June 2, 2010

Keywords: copper · DNA · EPR spectroscopy · nanomaterials · N,O ligands

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