Intrinsic Low Temperature Paramagnetism in B-DNA

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We present an experimental study of magnetization in λ-DNA in conjunction with structural measurements. The results show the surprising interplay between the molecular structures and their magnetic property. In the B-DNA state, λ-DNA exhibits paramagnetic behavior below 20 K that is nonlinear in an applied magnetic field whereas, in the A-DNA state, it remains diamagnetic down to 2 K. We propose orbital paramagnetism as the origin of the observed phenomena and discuss its relation to the existence of long range coherent transport in B-DNA at low temperature.

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It is now common knowledge that the electrical conduction in DNA is intimately linked to experimental factors such as molecules' base pair (bp) sequence, type of electrodes, surrounding counterions, and the number of water molecules [1–4]. The experimental accounts to date span a whole spectrum of conduction mechanisms, from insulators, semiconductors, and metals to proximity induced superconductors [5–8]. Magnetization is an alternative, noninvasive mean to probe the intrinsic electronic properties of matter, as the measurements do not require any electrode attachments. Unlike the intensive experimental efforts made in electronic transport in DNA molecules, their magnetization has been scarcely explored due to experimental difficulties such as the overwhelming presence of water. Basic questions about the intrinsic magnetic properties of DNA, such as the magnitude of its magnetic susceptibility, χ_g, have remained unclear. It is widely known that DNA is diamagnetic near room temperature, with a sizable anisotropy stemming from the presence of aromatic rings of the base pairs whose magnitude is comparable to that of benzene [9–11]. But how does the overall magnetic state of DNA depend on intrinsic parameters (molecular structure, bp sequence) as well as on extrinsic parameters such as counterion types? Does DNA magnetization depend on these parameters in a way reminiscent to the electrical conduction? And if so, what are the consequences and implications for the usage of DNA as molecular wires? To answer these questions, we have studied the low temperature susceptibility and magnetization of randomly oriented λ-DNA molecules and its relation to their molecular structure (A- and B-DNA) and counterion types (Na^+ and Mg^{2+}), two parameters known to greatly influence the electronic property of DNA [1]. We find that the magnetization is temperature independent and diamagnetic at high temperatures (100 K and above) regardless of water content in both A- and B-DNA structures. Surprisingly, once the molecules are sufficiently ''wet'' and thus are found in the B structure, a paramagnetic upturn is observed at lower temperatures that are nonlinear in magnetic field in addition to the atomic diamagnetic component. Collectively, these observations reveal, for the first time, the intrinsic nondiamagnetic state in DNA molecules that is intricately related to their molecular structures.

λ-DNA samples (400 μg each) in two counterion types, Na^+ (hereafter called NaDNA) and Mg^{2+} (MgDNA), were prepared in quartz capillary tubes that served as sample holders for both magnetization (QuantumDesign MPMS-R2 SQUID magnetometer) and structural studies (micro-Raman spectrometer) [12]. λ-DNA (16 μm, 48 502 bp) was chosen specifically because the proximity induced superconductivity and the low temperature negative magnetoresistive behavior were detected previously in these molecules. The molecular structure of DNA changes dramatically with surrounding hydration levels. In an aqueous environment, DNA molecules are in a B-DNA structure where base pairs are stacked parallel to one another with the inter-bp distance of 3.2 Å and the helix diameter of 19 Å. When molecules are dried, the bases become severely tilted off the helix axis and the helical diameter becomes broad (23 Å) [13]. The molecular structure of the samples was transformed between the dry A-DNA and the wet, natural B-DNA states by adding or removing water from the samples. In their driest states, NaDNA and MgDNA samples contained <0.1 and ~ 0.3 μl of H_2O, respectively. The corresponding values of the sample's relative humidity (RH) (the weight of H_2O divided by that of dry DNA) are 0.25 and 0.75. At each stage of rehydration and/or dehydration the quartz capillaries were sealed to maintain a constant water content, and the magnetization and the molecular structure (via micro-Raman spectroscopy) were studied in parallel. Figure 1(a) shows the magnetization (M) of NaDNA with <0.1, ~ 0.9 μl (RH = 2.25), and ~ 1.9 μl (RH = 4.75) of water as a function of temperature (T) at 5 T. Originally, the sample contained 0.9 μl of H_2O. Then the water content was increased to 1.9 μl and finally dried down to $\leq$ 0.1 μl. By subtracting the water contribution from the total magnetization at $T > 100$ K, we have extracted the diamagnetic susceptibility of DNA, $\chi_{\mathrm{DNA}} = -0.63 \pm 0.1 \times 10^{-6}$ EMU $\cdot$ G^{-1} g^{-1}. Within the experimental accuracy,

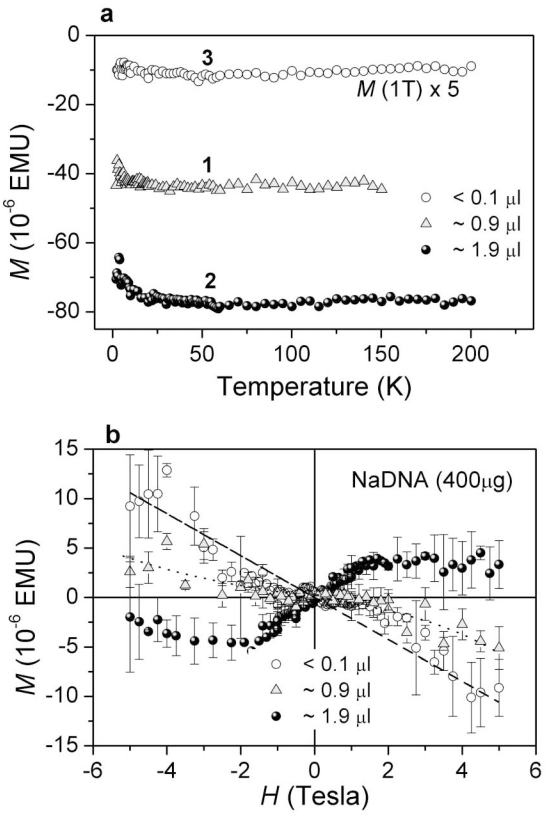

FIG. 1. Evolution of magnetization (M) of NaDNA with water content. (a) Magnetization of DNA and water as a function of temperature (T). Above 100 K, the change in M corresponds to the temperature independent diamagnetic contribution from H_2O. The numbers in bold letter indicates the order in which the measurements were taken. (b) Magnetization as a function of magnetic field (H) measured at 2 K without the water contribution (-7.2×10^{-10} EMU $\cdot$ G^{-1} μl^{-1}). The straight dashed line represents the diamagnetic component of NaDNA measured at 150 K. The dotted curved line superimposed on the data points with $H_2O = 0.9$ μl is a guide to the eye. Error bars represent the standard deviation in the measurements. Some error bars on low field data, $H \leq 1$ T, are removed in order to avoid crowding the graph. A ferromagnetic component originating from impurities in the quartz tube was detected whose magnitude saturates at $H \sim 7000$ G ($M \sim 5 \times 10^{-6}$ EMU for both NaDNA and MgDNA). This component was independent of temperature and of water content and has also been subtracted.

the diamagnetic susceptibility was found to be independent of water content, that is, $\chi_{\text{A-DNA}} = \chi_{\text{B-DNA}}$. This value, determined from two NaDNA samples, is in fair agreement with the calculated atomic diamagnetic susceptibility of DNA, $\sim -0.52 \times 10^{-6}$ EMU $\cdot$ G^{-1} g^{-1} [14]. At temperatures below 20 K, the magnetization of NaDNA containing 1.9 and 0.9 μl of H_2O indicates unexpected paramagnetic upturn that disappeared once the sample was dried to $H_2O < 0.1$ μl (RH = 0.25). Figure 1(b), portraying M (without H_2O contribution) as a function of magnetic field (H) at $T = 2$ K, clearly presents this low temperature paramagnetism. At helium temperature, DNA in an aqueous environment exhibits a crossing over from diamagnetic to paramagnetic magnetization that is nonlinear in magnetic field. The magnitude of this paramagnetic increase, $\Delta M_{\text{para}} = M_{\text{tot}} - M_{\text{dia}}$, is comparable to that of the diamagnetic contribution of DNA. The corresponding Raman spectra depicting the structural transformation from the A to the B structure in NaDNA are presented in Fig. 2 [15]. Among the large and highly reliable index of Raman bands corresponding to vibrational modes of DNA geometry and conformations [16], we concentrate on two bands representing the backbone vibrations to identify the structural state of our samples, as described in the figure caption. While with <0.1 μl of H_2O, the sample was found almost purely in the A state, both B-DNA and a small signature of A-DNA were observed with 0.9 μl (RH = 2.25). With a further addition of H_2O, molecules were found entirely in the B state. By comparing the molecular structure and the magnetization of NaDNA, it appears that B-DNA is a prerequisite condition for the low temperature paramagnetism in DNA. It needs to be noted, however, that the value of RH = 2.25 of our sample, for which the coexistence of A- and B-DNA is observed, exceeds the generally respected value of RH = 0.9 found in the literature, above which DNA is known to exist totally in the B state [17]. This observation indicates that H_2O is not diffused uniformly due to the sample geometry and the preparation method. The more rigorous investigation of the water content and the structural analysis of NaDNA samples via x-ray diffraction will be reported elsewhere.

In the MgDNA sample, we were unable to remove H_2O sufficiently to create a predominantly A-DNA state. In fact, the Raman spectra (not shown) of the MgDNA in its driest

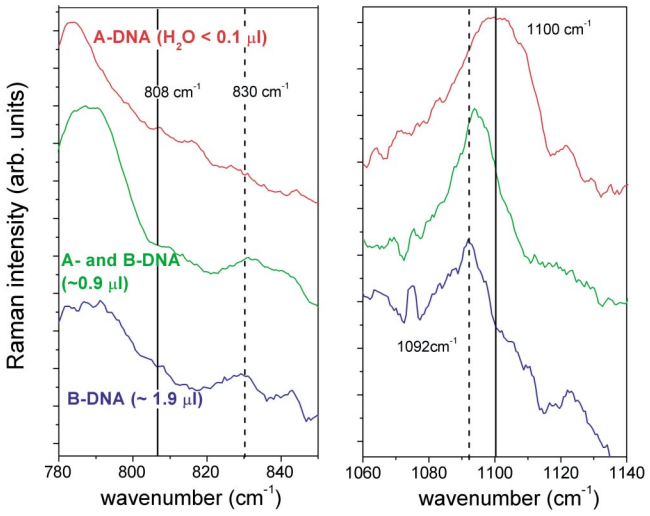

FIG. 2 (color). Evolution of Raman spectra on NaDNA: The left panel shows the evolution of Raman band corresponding to the complex vibrational mode of the backbone network along the chain (5′C-O-P-O-C3′). This band shifts from 807 cm^{-1} in A-DNA to 835 $\pm$ 5 cm^{-1} in B-DNA. The right panel shows the band associated with the symmetric stretching of the PO_2 moiety mode. This band shifts from 1100 cm^{-1} in A-DNA to 1092 cm^{-1} in B-DNA [16].

state (0.3 μl, RH = 0.75) indicated mainly B-DNA bands, and with ~0.5 μl (RH = 1.25) of H_2O the molecules were found to be in a purely B-DNA state. This is in marked contrast to NaDNA where the presence of both A- and B-DNA was detected at much higher water content. This observation is consistent with a known property of Mg^{2+} that prevents the transition from B- to A-DNA more efficiently than Na^+ ions [18]. The magnetization measurements on MgDNA with ~0.5 μl of H_2O preceded the measurements with 0.3 μl. As can be seen from Fig. 3, a purely diamagnetic behavior at low temperatures was never achieved in MgDNA in line with the observation in NaDNA. In the driest state, only a slight decrease in ΔM_{para} was detected. Furthermore, the paramagnetic magnetization was found to become independent of water content for H_2O values higher than 0.5 μl (measured up to 2.2 μl). The temperature dependence of the magnetization of wet MgDNA follows the Curie law, as shown in the inset. Susceptibility at higher temperatures was determined to be $-0.8 \pm 0.1 \times 10^{-6}$ EMU $\cdot$ G^{-1} g^{-1}, larger than the value found for the NaDNA sample. This difference ($\sim 0.2 \times 10^{-6}$ EMU $\cdot$ G^{-1} g^{-1}) corresponds to the diamagnetic susceptibility of the residual buffer ions ($MgCl_2$ and NH_4 acetate). It is also noteworthy that the low temperature ΔM_{para} was found to be ~4 times higher in MgDNA than in NaDNA.

The apparition of paramagnetism in B-DNA at low temperature that is nonlinear in applied field is robust. There are two possible origins for the observed nonlinear paramagnetism: electron spin (s) or orbital magnetism. Assuming the electron spins (magnetic ions or hydroxyl radicals [19], for example) render the observed behavior, we have fit the paramagnetic component of the magnetization, ΔM_{para}, to the Brillouin and Curie law. The best fits were obtained for $s = 1/2$–$3/2$, with the total number of spins of ~10^{15}, or 1-spin/400 bp, for NaDNA and ~4 × 10^{15}, or 1-spin/100 bp, for MgDNA, respectively. Such high concentrations of spins should be detectable by electron paramagnetic resonance (EPR), provided that the signal line width does not exceed 300 G. The examination via EPR [20] revealed no such presence in NaDNA at room temperature. The MgDNA sample was examined between room temperature and 4 K. The only EPR absorption signal was detected at $g = 4.28$, which can be attributed to Fe^{3+} ($s = 5/2$, $g = 30/7$) in an asymmetric crystal field. The number of these spins was determined to be of the order of 10^{12}, far too small to be responsible for the observed ΔM_{para} [21]. Furthermore, the magnetization of H_2O used to humidify the samples was examined separately using the SQUID magnetometer. ΔM_{para} (5 T, 2 K) less than 10^{-6} EMU was found in 2 μl of H_2O. Therefore these experiments, as well as the disappearance of paramagnetic component in A-DNA after the drying process, exclude free radicals and magnetic impurities in water and buffer solutions from the possible origins of low temperature paramagnetism, as such impurities cannot be removed from the sample by a simple evaporation of water.

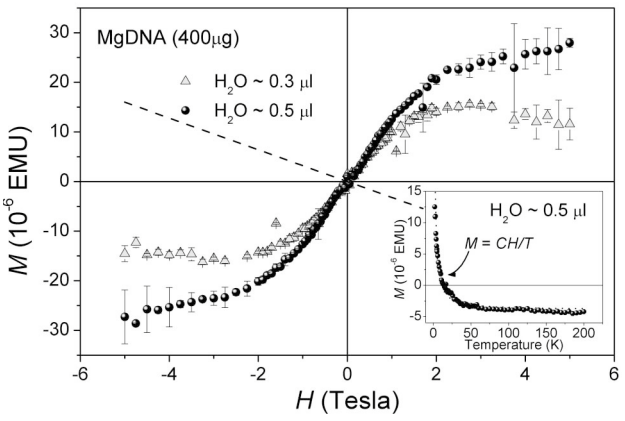

FIG. 3. M vs H of MgDNA measured at $T = 2$ K. The data presented here are treated in the same manner as in Fig. 1(b). Inset: M as a function of temperature of wet MgDNA at $H =$ 1 T. Dotted line represents the fit to the Curie law, $M(T) = C \times H/T$, with C (the Curie constant) $= 4.3 \pm 0.2 \times 10^{-9}$ J $\cdot$ K/T^2 corresponding to ~10^{15} of 1/2 spins.

Thus far, the paramagnetism appears to be an intrinsic property unique to B-DNA. An interesting possibility is the existence of persistent current loops along the DNA molecules on a mesoscopic micron scale. The mesoscopic orbital magnetism has been shown theoretically to be paramagnetic and nonlinear when repulsive electron-electron interactions dominate over single particle effects [22,23]. The total magnetization of the system then follows $m(H) = [\chi_L k_F \sqrt{S(T)} H]/[1 + (\frac{HS(T)}{\phi_0})^2]$, where χ_L is the Landau susceptibility, and k_F is the Fermi wave number. The nonlinear magnetization reaches its maximum at $H_0 = \Phi_0/S(T)$, where $\Phi_0 = h/e = 4.14 \times 10^{-7}$ G cm^2 is the magnetic flux quantum and $S(T)$ is the maximum surface area enclosed inside the coherent current loop [Figs. 4(a) and 4(b)]. The orbital magnetism associated with the persistent currents has been already observed in mesoscopic rings and 2D squares and is considered the hallmark of phase coherent transport at low temperatures [24]. In our DNA samples measured at 2 K, the magnitude of the paramagnetic signal is 2–4 times that of the total diamagnetic susceptibility. At lower temperatures the size of orbital paramagnetic susceptibility is expected to grow rapidly. We estimate the electron coherent length, L, using the experimental values from our measurements, $H_0 =$ 1 ~ 2 T, via $S(T) = d \times L(T)$, where d is the distance between bases of B-DNA molecules. Our calculation yields the electron path on the order of 1 μm along the helical axis of the molecules. Such circulation of electrons enclosing a finite flux can be achieved through the combination of intra- and interstrand (across the hydrogen bonds) transfer of π electrons on bases [Fig. 4(c)]. Hydrogen bond assisted electronic exchange has already been witnessed in some organic molecules [25]. The value of the electron path found here agrees with the coherent length of ~1 μm in λ-DNA, determined by Kasumov *et al.* [3] where prox-

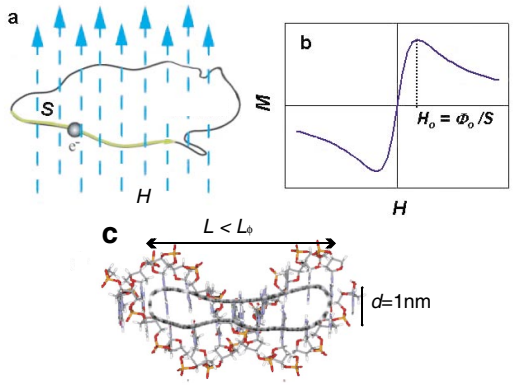

FIG. 4 (color). (a) Schematic view of a mesoscopic ring flux in perpendicular magnetic field H. (b) Shape of orbital magnetization as a function of magnetic field in the nonlinear regime. (c) Simplified picture of a persistent current path inside and along the helical length of a B-DNA molecule involving interstrand charge transfer.

imity induced superconductivity was detected at $T < 1$ K. Our observation may also imply a coherent electron transport along the helical length of the molecule at low temperatures, but exclusively in B-DNA, consistent with experimental reports on the DNA electronic conductivity that showed higher conductivity in wet-DNA molecules [26,27]. Last, from the enhanced size of the low temperature paramagnetic signal as well as the persistence of B-DNA in MgDNA, Mg^{2+} appears to facilitate the electron transfer inside DNA molecules.

In summary, we have found a low temperature, nonlinear paramagnetic behavior in the B state of λ-DNA molecules. This effect is found in both DNA samples prepared with Na^+ and Mg^{2+} counterions. The paramagnetic susceptibility of molecules prepared with Mg^{2+} ions is found larger by a factor of 4 compared to the Na^+ counterpart. The present results can be interpreted by the existence of a mesoscopic orbital paramagnetism in B-DNA molecules that may be related to the proximity induced superconductivity observed in these molecules. Magnetization of other types and of aligned DNA molecules should be examined in order to confirm the orbital origin of this paramagnetism.

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[1] R. G. Endres *et al.*, Rev. Mod. Phys. **76**, 195 (2004).
[2] Ch. Adessi *et al.*, Comp. Nanosci. Nanotech. **2002**, 56 (2002).
[3] A. Yu. Kasumov *et al.*, Appl. Phys. Lett. **84**, 1007 (2004).
[4] S. O. Kelley and J. K. Barton, Science **283**, 375 (1999).
[5] P. J. de Pablo *et al.*, Phys. Rev. Lett. **85**, 4992 (2000).
[6] H. W. Fink and C. Schönenberger, Nature (London) **398**, 407 (1999).
[7] T. Porath *et al.*, Nature (London) **403**, 635 (2000).
[8] A. Kasumov *et al.*, Science **291**, 280 (2001).
[9] G. Maret *et al.*, Biopolymers **22**, 2727 (1983).
[10] G. Maret *et al.*, Phys. Rev. Lett. **35**, 397 (1975).
[11] E. Iizuka and Y. Kondo, Mol. Cryst. Liq. Cryst. **51**, 285 (1979).
[12] Samples were obtained from New England Biolabs and Amersham Bioscience (500 μg/ml with 10 mM Tris-HCl and 1 mM EDTA). NaDNA: The original solution was diluted in 10 ml of 60/40 H_2O/Isopropanol solution containing 0.3 M of NaCl for coprecipitation. The solution was then centrifuged at 15 kG and at 4 °C for 35 min. The precipitate was rinsed in 70% ethanol and was centrifuged again at 15 kG and at 4 °C for 15 minutes. This procedure was repeated twice to remove the excess Na^+ ions. MgDNA: The original solution was replaced by 9 mM $MgCl_2$/20 mM NH_4-acetate buffer solution by dialysis. This solution was concentrated via centrifuging through a Microcon (Millipore) filter, down to ~5 mg/ml, then lyophilized for 2 h. The H_2O content of the samples were controlled by injection (Milli-Q distilled and deionized H_2O, >18 MΩ) or by evaporation at 45–55 °C (~48 h for NaDNA and over 1 week for MgDNA samples). Quartz capillary tube sample holders were obtained from Heraeus (2 mm o.d. and 1 mm i.d.). The H_2O amount was determined using a precision scale (± 0.1 μg). Samples were suspended by capillary force at the midheight of the tube.
[13] A- and B-DNA structures can be found in, for example, K. E. van Holde, *Chromatin*, Springer Series in Molecular Biology (Springer-Verlag, Paris, 1988).
[14] The published magnetic susceptibility values [cf. *CRC Handbook of Chemistry and Physics* (CRC Press, Inc., Boca Raton, FL, 1983), 64th ed.] of individual atoms found in natural DNA molecules were used for the calculation.
[15] 514.5 nm excitation line of an Ar^+-Kr^+ laser was used in a confocal micro-Raman configuration with ×10 magnification. The scattered light was analyzed using a Jobin-Yvon triple grating spectrometer (T64000) consisting of a holographic notch filter and liquid nitrogen cooled CCD detector. The radiation power at source was between 3 and 10 mW. The spectra were taken at several regions within the sample. Spectra shown here are the accumulated averages of 10 exposures of 30–60 s each. The effective spectral resolution was less than 1 cm^{-1}.
[16] H. Deng *et al.*, Biopolymers **50**, 656 (1999).
[17] S. M. Lindsay *et al.*, Biopolymers **27**, 1015 (1988).
[18] J. Schultz *et al.*, Biophys. J. **66**, 810 (1994).
[19] M. G. Debije *et al.*, Radiat. Res. **154**, 163 (2000).
[20] A Bruker spectrometer equipped with an Oxford cryostat was operated at 10 GHz operation frequency, 100 kHz modulation frequency, and 20 G modulation amplitude.
[21] The number of spins was determined by comparing the intensity of the spectra to that of a calibrated $CuSO_4 \cdot 5H_2O$ single crystal.
[22] B. L. Altshuler *et al.*, Phys. Rev. Lett. **66**, 88 (1991).
[23] V. Ambegaokar and U. Eckern, Phys. Rev. Lett. **65**, 381 (1990).
[24] L. P. Lévy *et al.*, Physica (Amsterdam) **189B**, 204 (1993).
[25] J. R. Ferrer *et al.*, Chem. Mater. **13**, 2447 (2001).
[26] P. Tran *et al.*, Phys. Rev. Lett. **85**, 1564 (2000).
[27] Y. Otsuka *et al.*, Jpn. J. Appl. Phys. **41**, 891 (2002).