PHYSICAL REVIEW B **75**, 121103(R) (2007)

Optical spectra of wet and dry *M*-DNA

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(Received 19 December 2006; revised manuscript received 23 February 2007; published 29 March 2007)

We present measurements of optical absorption spectra of *M*-DNA, a form of DNA where divalent metal cations are incorporated into the DNA structure by replacing one of the hydrogens from hydrogen bonds in the interior of a double helix. In our experiment we have used Zn^{2+} cations which readily form a complex with DNA in alkaline buffers (pH 9). Absorption spectra of wet and dry Zn-DNA are almost identical and show a redshift of absorption maxima. The red energy shift indicates an increase of the π-π overlap of adjacent molecular orbitals in a more compact *M*-DNA structure. However, the effect is smaller ($\sim$0.1 eV) than predicted ($\sim$1 eV) which we attribute to sequence randomness of native DNA used in the experiment.

DOI: 10.1103/PhysRevB.75.121103 PACS number(s): 87.14.Gg

Electronic properties of DNA have been studied since the mid 1950s.[1] Very soon it was found that DNA, which is colorless in aqueous solutions, absorbs strongly in the near uv region with an absorption maximum centered at around 260 nm. Numerous experimental[2–7] and theoretical[8–12] works have been published explaining the electronic transitions which lie behind the 260 nm absorption peak. The transitions are intrabase (localized on a particular DNA nucleobase) and are predominantly of π-π^* character. Interactions between planar bases stacked in a parallel fashion along the DNA double helix have only a secondary effect on shape, intensity, or spectral position of DNA optical absorption spectra.[13] Measurements of temperature dependence of dc conductivity on bulk DNA samples[14] revealed an activation energy of 2 eV which was absent in the absorption spectra but nevertheless it was a first indication for possible semiconductor behavior of DNA where molecular orbitals of adjacent nucleobases would form an effective π-way for electronic conduction. More than 3 decades later, in the late 1990s the idea was reborn in the realm of nanotechnology and many experimental results on electric conductivity of single DNA molecules[15–19] or bundles[20,21] have started to appear. Results for the DNA electrical conductivity were rather discrepant, presumably due to different experimental conditions, e.g., kinds of DNA samples (short/long, synthetic/native), ambient or vacuum conditions, contacts, etc., ranging from insulating to metallic. For a recent review see Ref. 22. Similarly, theoretical models for the DNA conductivity are also various: band resonant tunneling,[23] superexchange,[24] thermally induced hopping,[25] or polaronic.[26] Eventually, it becomes more and more accepted that DNA is not a good conductive material. A logical step towards achieving this goal has been to modify DNA in order to improve its conducting properties. One of the first approaches was to use DNA as a template for growth of metallic wires.[27] That attempt was successful but at the expense of losing important DNA properties—recombination and self-recognition. Another approach which would preserve those properties was to intercalate metallic cations directly into the DNA structure.[28] The intercalation works particularly well in high pH with Ni^{2+}, Co^{2+}, and Zn^{2+} cations. It was proposed that the intercalated cation replaces an imino proton of guanine (in guanine-cytosine base pairs) or thymine (in adenine-thymine base pairs). However, all attempts to crystallize *M*-DNA and to determine its crystal structure have been unsuccessful since Ref. 29. The new form was named *M*-DNA. A drastic improvement of electrical conductivity of *M*-DNA in comparison to native *B*-DNA has been indirectly demonstrated with a fluorescence quenching experiment[30] as well as by direct measurement of dc conductivity.[31] Very recently, density functional theory (DFT) simulations have shown that incorporation of some divalent metal cations into the DNA structure could significantly decrease its highest occupied molecular orbital (HOMO)-lowest unoccupied molecular orbital (LUMO) gap.[32]

The aim of our work was to investigate how intercalation of Zn^{2+} ions into the DNA double helix alters the DNA electronic structure and how it reflects in its optical absorption spectrum. In the first approximation, the optical absorption spectrum of DNA could be considered as a sum of the absorption spectra of the constituent nucleobases: adenine, guanine, thymine, and cytosine.[13] The absorption maxima corresponding to their first π-π^* transitions are centered around 4.6 eV. They are blueshifted from the 0-0 transition line due to the Frank-Condon effect.[13] However, a closer look on the absorption spectrum could reveal significant differences which reflect interactions between nucleobases stacked in a double helix structure of DNA.[33] Generally, interactions between chromophores, either of the same kind or different, could be divided into Coulombic and short range interactions.[34] The leading term in the Coulombic interactions is the dipole-dipole one and it is usually the only term considered in the excitonic model of interchromophore electronic interactions and excitation transfer. The dipole-dipole interactions depend on mutual orientations of interacting dipoles. In the case of DNA, strong π-π^* transition dipole moments are polarized in a plane of the nucleobases, i.e., perpendicular to the DNA molecular axis. Consequently, the absorption peak which corresponds to the first π-π^{*-} transitions in the DNA is less intense (by $\sim$40%) than the sum of the nucleobases absorptions peaks (the effect of hypochromism). The second effect of the dipole-dipole interac-

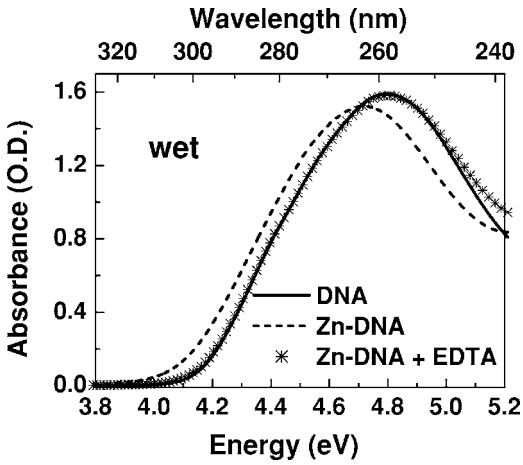

FIG. 1. Absorption spectra of pristine DNA (—), Zn-DNA with all intercalating sites occupied with Zn^{2+} (---), and Zn-DNA with added EDTA which removes Zn^{2+} cations from DNA (*) in solution.

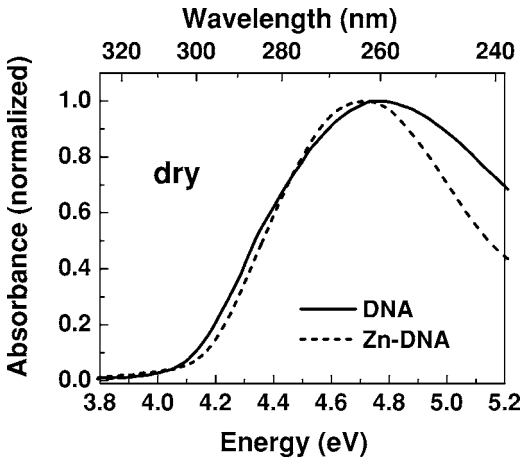

FIG. 2. Normalized absorption spectra of dry, *A*-DNA (—) and dry Zn-DNA (---).

tions on the DNA absorption spectra is a slight blueshift compared with the nucleotides absorption spectra, as explained in detail in Ref. 33. It has been readily observed in DNA dimers,[35] and single[36,37] and double stranded[33,38] DNA. The short range interactions—the charge density interpenetration and the quantum mechanical exchange interaction—both depend on interchromophore orbital overlap.[34] They play only a minor role in the DNA structures due to a weak orbital overlap but, when present, they would cause a redshift of the absorption spectrum.[34,39]

In our experiment we used native DNA from calf thymus (Sigma) and zinc chloride, $ZnCl_2$ (Sigma) as a source of zinc ions. DNA was dissolved in 50 mM tris-HCl buffer, pH 9 in concentration 100 μg/ml. A concentrated solution of $ZnCl_2$ in the same buffer was added into the DNA solution in increments which correspond to 0.3 Zn^{2+} per base pair (bp). The level of DNA saturation with Zn^{2+} was controlled by an ethidium bromide (EB) fluorescence assay: namely, when intercalated into DNA, EB fluoresces about 25 times more strongly than free in solution. Insertion of Zn^{2+} into the DNA structure precludes the EB intercalation thus making the EB fluorescence a direct measure of percentage of (un)occupied intercalation sites. A control sample was made by addition of ethylenediaminetetraacetic acid (EDTA) which is a well-known chelate ligand that strongly binds metal cations. When added to the DNA+$ZnCl_2$ buffer solution EDTA binds all Zn^{2+} ions in the solution including those which were intercalated into DNA. The reaction is immediate and could be readily observed in a strong increase of EB fluorescence. Hence by adding EDTA we could recover a native *B*-DNA from *M*-DNA. Optical absorption measurements of samples in solution were made in a standard 1 cm quartz cuvette. Dry samples were made by depositing 1 ml of solution on a quartz window and drying it overnight in a dessicator. Optical absorption spectra of both solutions and dry samples were measured with a HP 8453 spectrophotometer.

In Fig. 1 we show absorption spectra of calf thymus (CT) DNA, Zn-DNA, and Zn-DNA with EDTA in solution. The absorption maximum of CT DNA spectrum is centered at 4.80 eV (258.6 nm). It undergoes a redshift upon intercalation of Zn^{2+} ions into the DNA structure to a value of 4.71 eV (263.3 nm). It is also about 5% less intense then the spectra of pristine DNA. Subsequent addition of EDTA, which removes Zn^{2+} from DNA, restores the initial absorption curve confirming that intercalation of Zn^{2+} is the sole reason for the redshift of the absorption maximum. The redshift is less visible for dry samples (Fig. 2) because the absorption of pristine DNA itself shifts to lower energies; 4.78 eV (259.3 nm), and also broadens when DNA transforms from its wet, *B*-form to a dry, *A*-form (Fig. 3). Differences in absorption spectra of *B*-DNA and *A*-DNA can be explained as a consequence of transition from the more ordered *B*-structure to the more disordered *A*-structure. As stated, the dipole-dipole interactions depend on mutual orientations of interacting dipoles and are effective only in ordered structures of dipoles.[40] In our case they cause a blueshift of the ordered *B*-DNA absorption spectrum compared to the spectrum of the more disordered *A*-DNA. Contrarily, the absorption spectra of wet and dry Zn-DNA (Fig. 4) are almost indistinguishable and both are centered at 4.71 eV. Thus we could conclude that *M*-DNA does not change its

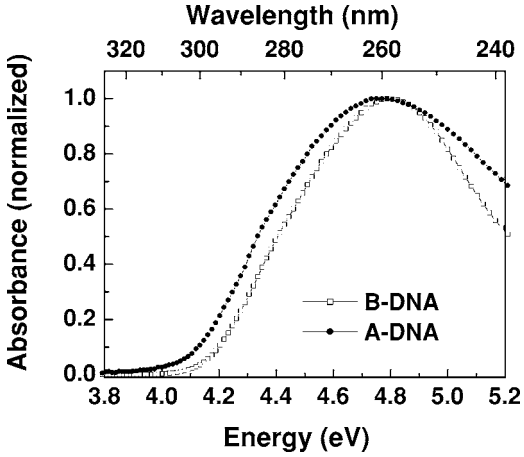

FIG. 3. Comparison of normalized absorption spectra of wet, *B*-DNA (open squares) and dry *A*-DNA (closed circles).

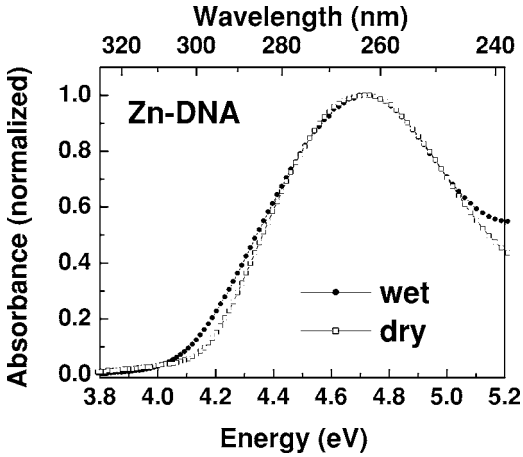

FIG. 4. Comparison of normalized absorption spectra of wet (open squares) and dry (closed circles) Zn-DNA.

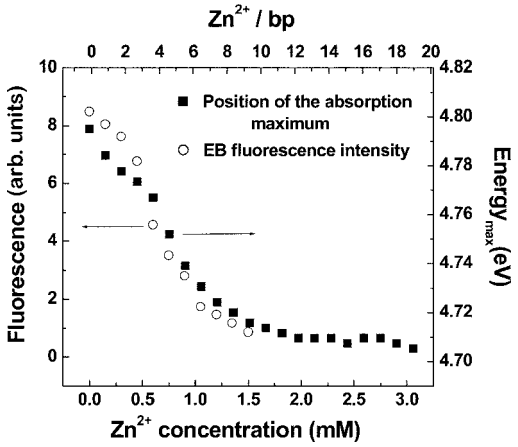

FIG. 5. Position of absorption maxima (in eV) as a function of Zn^{2+} concentration (closed squares, right scale). The curve saturates at Zn^{2+} concentrations >1 mM after all intercalation sites have been occupied by cations as confirmed with EB fluorescence assay (open circles, left scale). The top scale measures the cation concentration in number of Zn^{2+} per base pair.

structure significantly upon drying. Although it is believed that the *B* to *M* transition is cooperative,[28] we have found that the transition is gradual (at least for CT DNA) as shown in the continuous increase of the redshift with Zn^{2+} concentration (Fig. 5) until it reaches a value (>1 mM) at which all intercalation sites are occupied as detected with EB fluorescence. Possible reasons for gradual behavior are length and sequence randomness of native CT DNA, and a fact that DNA fragments with different sequences undergo the transition at different Zn^{2+} concentrations.[28] The final result is a quasicontinuous transition from the *B*-form to the *M*-form which completes at Zn^{2+} concentration corresponding to about ten zinc cations per base pair.

Origins of the small, but significant changes in optical absorption spectra of *M*-DNA could be found in its structural differences, compared to the canonical *B*-DNA. Measurements of mobility in a gel electrophoresis experiment have suggested that the double helix of *M*-DNA is shorter and more compact then that of *B*-DNA.[28] Similarly, DFT calculations[32] also indicated that insertion of metal cations deforms DNA structure such that distances between neighboring bases in the DNA stack became slightly shorter. They also predict changes in the double helix twist, propeller twist, and tilt angels of planar nucleobases which would additionally enhance the π-π overlap of molecular orbitals. Considering a shorter interchromophore distance we could expect that dipole-dipole interactions are even more effective in *M*-DNA than in *B*-DNA. That could be a plausible explanation for the hypochromic effect (a decrease of the absorption peak intensity) but not for the *red*shift. As mentioned above, enhanced dipole-dipole interaction would induce a *blue*shift. Therefore the only reason for the redshift of the absorption maximum could be an enhancement of the orbital overlap. Since CT DNA has a random sequence of base pairs an effective overlap increase could be expected only for short segments in the DNA sequence where nucleobases of the same kind face each other. This could be a possible reason why the observed redshift of the absorption maximum ($\sim$0.1 eV) is much smaller then the theoretically predicted decrease in the HOMO-LUMO optical gap ($\sim$1 eV).[32]

In conclusion, measuring optical absorption spectra of Zn-DNA in a solution and in a dry form and comparing them with corresponding spectra of pristine DNA we have found that the absorption maximum shifts to lower energies by $\sim$0.1 eV. We argue that the shift is caused by structural changes in the *M*-DNA double helix induced by the intercalated metal cation. Those structural changes enhance the orbital π-π overlap between molecular orbitals of neighboring nucleobases and consequently increase the exchange interaction between them. We suggest that the absorption maximum shift is smaller then predicted because of sequence randomness in the native CT DNA, where only certain fragments of uniform base-pair sequences exhibit an effective increase of the π-π stacking. As a corollary, it should be expected that formation of *M*-DNA would have a stronger effect on the electronic structure of DNA homopolymers such as poly(dG)-poly(dC) or poly(A)-poly(dT).

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