Strong coupling between adenine nucleobases in DNA single strands revealed by circular dichroism using synchrotron radiation

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Circular dichroism (CD) experiments on DNA single strands (dA_n) at the ASTRID synchrotron radiation facility reveal that eight adenine (A) bases electronically couple upon 190 nm excitation. After $n=8$, the CD signal increases linearly with n with a slope equal to the sum of the coupling terms. Nearest neighbor interactions account for only 24% of the CD signal whereas electronic communication is limited to nearest neighbors for two other exciton bands observed at 218 and 251 nm (i.e., dimer excited states). Electronic coupling between bases in DNA is important for nonradiative deexcitation of electronically excited states since the hazardous energy is spread over a larger spatial region.

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The "communication" between different nucleobases in a DNA molecule is of great importance in various research fields and has spurred the interest of both theorists and experimentalists. In molecular electronics, electronic coupling between nucleobases is the basis for the use of DNA as a conducting nanowire [1], though opinions vary from DNA being an insulator to being a semiconductor to a good conductor [2–4], and under some conditions even a superconductor [5]. Biologically, damage to DNA by free electrons may not be located on the initially charged nucleobases due to large electronic coupling between bases [3,6]. Also the nature of excited states of DNA bases is important for the photostability of DNA with respect to uv damage. One protection mechanism is fast nonradiative relaxation to the electronic ground state [7], and another is delocalization of the excitation energy over several nucleobases to prevent subsequent photochemical reactions [8,9]. Therefore the electronic coupling between nucleobases in DNA is essential for nonradiative deexcitation of electronically excited states [8,9]. The formation of excimers in which the excitation energy is shared between two stacked bases limits the excitation energy to one strand at a time, leaving the other strand undamaged. The actual number of bases that couple in the excited state is therefore an important parameter, but hard to measure, and our current knowledge relies to a large extent on theoretical models [9–12]. In general, a proper description of electron delocalization is a very complex problem that depends on the base sequence and the folding motif of the strand (base stacking and base pairing).

Whether the singlet excited states are localized on a single base or delocalized over several bases has been under much debate [13–15]. In this work, we use circular dichroism spectra recorded at the ASTRID synchrotron radiation facility in Aarhus [16] to show that the electronic coupling between adenine bases is to a good approximation limited to two nucleobases for excitation wavelengths above 200 nm, but extends up to about eight bases for the electronic transition at 190 nm. The advantage of synchrotron radiation for such experiments is the large available photon fluxes in the vacuum ultraviolet (vuv), where absorption is strong. We believe that this is the first time information on the spatial electronic coupling has been provided by vuv excitation.

Absorption and circular dichroism spectroscopy of oligonucleotides in aqueous solution at neutral *p*H was carried out to probe the evolution of the signals as a function of length of the oligonucleotide. The samples used were (adenosine 5′-monophosphate) (dAMP) and oligonucleotides of adenine (dA_n with $n=2-30$) that form stacked single-helical structures at neutral *p*H in *B*-type DNA conformation [17–20].

All the oligonucleotides were purchased from DNA technology, Aarhus, and the mononucleotides from Sigma-Aldrich. The concentrations were determined using the calculated extinctions coefficients at 260 nm by the nearest neighbor method [21,22]. The nucleotides were dissolved in 10 m*M* phosphate buffer at *p*H 7.4 and 100 m*M* of NaF. However, the presence of salt did not affect our results and neither did the temperature (between 5 and 85 °C). The typical concentration of the oligomers was 5 m*M* for the monomers and ranged between 0.5 and 0.1 m*M* for the longer ones. A quartz cell type QS124 with a path length of 0.1 mm (Hellma GmbH, Germany) was used for the measurements. The experiments were performed at the UV1 beamline at the ASTRID synchrotron radiation source at University of Aarhus, Aarhus, Denmark. The same setup was used for measuring the absorption and circular dichroism (CD) spectra. The details of the setup are given elsewhere [23,24]. The range of wavelengths used was between 170 and 330 nm.

Spectra of dAMP and dA_2 are shown in Fig. 1(a). The absorption in the vuv region is significantly larger for the dinucleotide than for the mononucleotide. This we ascribe to the influence of one adenine molecule on the other to give a mixed dimer state, that is, the excited states of the dimer are linear combinations of the excited states of each monomeric chromophore. The result is two new states that are shifted in energy by equal amounts from the excitation energy of the monomer. Evidence of such exciton coupling becomes even clearer in the CD spectrum of dA_2 from the strong negative and positive bands at 182 and 194 nm with nodal point in between at about 188 nm. The CD signal of dAMP in this

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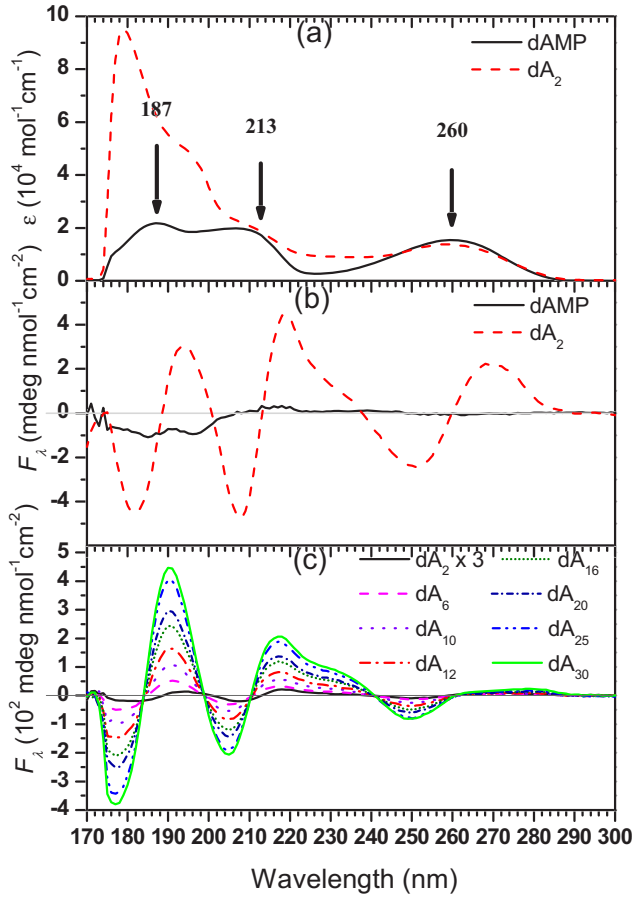

FIG. 1. (Color online) (a) Absorption per nucleobase of dAMP and dA_2.(b) CD spectra of dAMP and dA_2. (c) CD spectra of dA_n.

region is much smaller. Nodal points at about 213 and 241 nm are also signatures of two other exciton splittings in dA_2. It appears that an exciton node is present at 260 nm for dA_2, but a comparison with the higher-polymer CD spectra reveals that the node is at 241 nm with the positive exciton band mixed with the positive exciton band centered on 213 nm [Fig. 1(b)]. We note that these spectra are similar to previously published spectra [25,26], and to the pioneering measurements of Johnson and Tinoco that showed significant coupling between adjacent bases in a stack [27].

No new spectral features appear upon an increase in the length of dA_2 up to $n=30$ [Fig. 1(c)], except for a change in the magnitude of the signals and a small shift of the band positions. This finding indicates that no new states contribute to the CD. The nature of the transitions at 190, 218, and 251 nm is explored by considering how they depend on the size of the oligomer. First, the CD signals at 218 and 251 nm increase linearly with n with a slope close to the signal of dA_2 [Fig. 2], which indicates that only nearest neighbor interactions between adenines play a role for the coupling in the excited state. On the other hand, we observe that the signal at 190 nm is nonlinear up to about $n=8$, and after that it becomes linear [Fig. 3(a)]. Hence a maximum of about eight adenines electronically couple in the excited state, which implies that coupling extending over more than one helix turn is insignificant. The corresponding negative band at 177 nm also shows the same trend, but the data at that

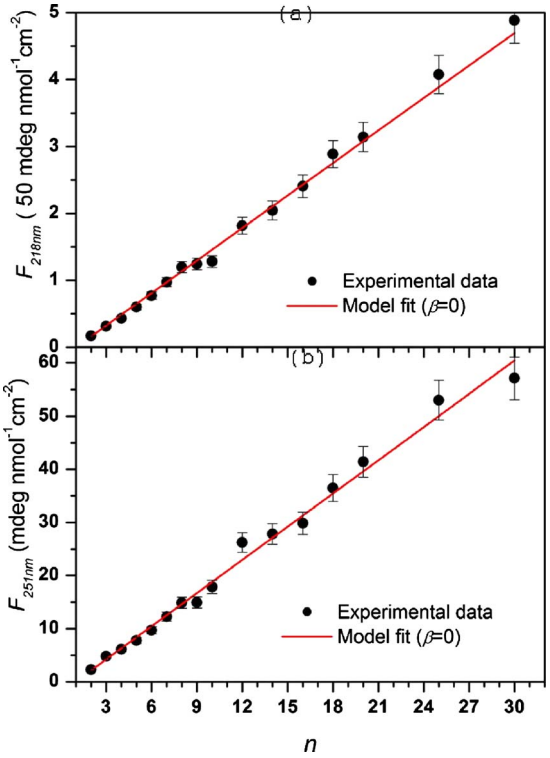

FIG. 2. (Color online) CD signal of dA_n plotted against n. Excitation wavelength 218 (a) and 251 (b) nm. The model fits are linear fits with $\beta=0$ corresponding to the inclusion of only nearest neighbor interactions in the model described in the text [Eq. (2)].

wavelength are less reliable due to strong absorption in water and as a result a low photon flux.

In order to explain the observed nonlinear behavior in the CD at 190 nm, we invoke a simple model. We decompose the coupling into nearest neighbor, next-nearest neighbor, next-to-next nearest neighbor and so on. For example, the CD signal (F_λ) of dA_3 can be expressed as twice the dimer signal plus a term due to coupling between all three adenines, and so on. This can be written as

$$F_\lambda(n) = \sum_{i=1}^{n} a_i(n-i) \tag{1}$$

where a_i are coupling elements. We now assume that a_i falls off exponentially with distance,

$$a_i = \alpha \exp(-\beta i d) \tag{2}$$

where d is chosen to be the distance between two nucleobases (3.4 Å). From a fit to the 190 nm data, a β value of 0.082 $Å^{-1}$ was obtained [Fig. 3(a)]. The nearest neighbor coupling as given by a_1 contributes only 24% $[a_1/(a_1+a_2+a_3+\cdots)]$ to the total coupling $(a_1+a_2+a_3+\cdots)$; in other words, 76% of the signal is due to higher-order couplings $(a_2+a_3+a_4+\cdots)$. If a fit were done only to the high-n data, extrapolation to low n would result in too low CD signals or even negative values, since nonexisting coupling terms would be included. In the case of the 218 and 251 nm bands, the slope is equal to a_1, and the total signal is satisfactorily

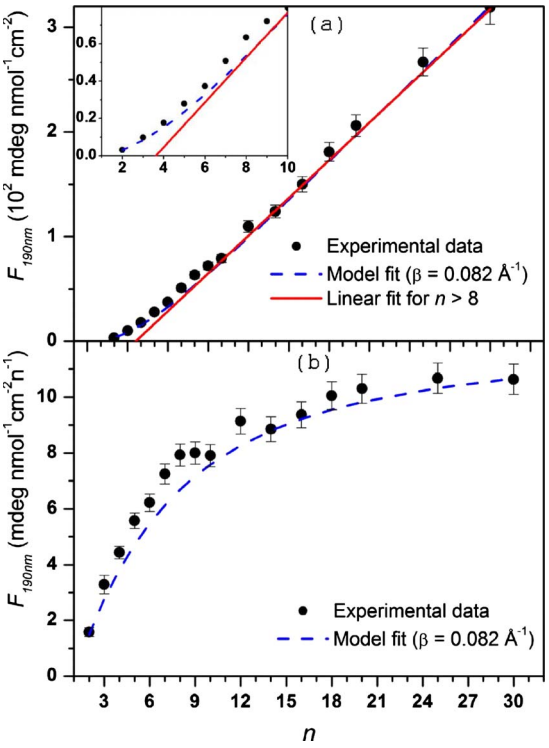

FIG. 3. (Color online) (a) CD signal at 190 nm. The line is a fit to points corresponding to $n>8$ and the dashed line is a fit to a model described in the text. The inset displays the plot for lower n values. (b) Same as (a) with values divided by n.

described by only nearest neighbor interactions.

Recent time-resolved fluorescence spectroscopy with femtosecond resolution of DNA oligomers suggested that excited states are delocalized over several bases [28] and theoretical calculations predicted electron delocalization over at least two bases [10–12]. Furthermore, time-resolved transient absorption experiments pointed to the formation of adenine excimers upon uv excitation [8]. Very recent femtosecond time-resolved broadband spectroscopy experiments by Buchvarov *et al.* [29] revealed a $1/e$ delocalization length for dA_n of 3–4 bases at 270 nm excitation, significantly larger than the coupling between only two bases found from our measurements in the uv. However, such a state (dark state) is likely to be different from the initially excited state (bright state) which is the subject of our study. Clearly, more experiments are needed to address the actual pathway after excitation, such as nuclear motions.

We also carried out an experiment on oligonucleotides of thymine, dT_n, and found in this case only dimer interactions in the vuv (177 nm). Indeed, thymine is known to have the lowest stacking interaction among the nucleobases. When thymine is between two adenine molecules in the sequence, the adenine coupling is also significantly lowered [30].

In conclusion, we have shown that the spatial extent of electron delocalization in DNA single strands of adenine highly depends on the energy of the excited state: at 190 nm excitation, eight adenines electronically couple, whereas the coupling is limited to two bases for excitation at 218 and 251 nm. From the perspective of a self-protection mechanism, excitation at high photon energies requires large electron delocalization, which according to our experimental results indeed is the case for DNA of adenine.

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