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Effect of introducing thymine spacers into an adenine strand: Electronic decoupling?

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ABSTRACT

Electronic coupling between DNA bases governs the deexcitation pathways after light absorption as well as the ability of the DNA strand to conduct charge. UV excitation of single strands of adenine bases involves two adjacent bases while the spatial extent of the excited state wavefunction following VUV excitation is over eight bases. In this work, we have recorded synchrotron radiation circular dichroism spectra for a series of DNA strands on the form $A_nT_mA_n$, $n = 1–5$ and $m = 1–3$, in aqueous solution to study the effect of introducing thymine spacers on the electronic coupling between the adenines. We find that a single thymine spacer is enough to eliminate the strong coupling between the adenine bases for all excitation wavelengths between 175 nm and 330 nm.

1. Introduction

The extent of the excited state wavefunction or the electronic coupling between bases in DNA strands is of great importance in several research areas. In the field of molecular electronics, DNA strands have a strong potential as electrical conductors, *i.e.*, nanowires [1–5]. Indeed, electron hopping depends on the strength of the π-stacks and the presence of delocalized domains, which is governed by DNA conformation and base sequence [1]. From the perspective of photobiology the photostability of DNA depends on the nature of the initially excited state after absorption of ultraviolet light and its coupling to other states. Several protection mechanisms are proposed in the literature. One is fast nonradiative relaxation to the ground state [6] and another is delocalization of the excitation energy over several bases to minimize damaging photochemical reactions [7–9].

Circular dichroism (CD) is a powerful tool to study the excited-state physics of DNA. This spectroscopy technique provides direct information on the electronic coupling between bases since the CD signatures differ significantly between a monomer and a dimer (exciton coupling band) [10]. CD is typically carried out in the ultraviolet (UV) region but useful information is also obtained in the vacuum ultraviolet (VUV) [10,11].

In Aarhus, we take advantage of the synchrotron radiation (SR) facility ASTRID as a source of high fluxes of UV and VUV photons. An intense light source is particularly important below 200 nm where absorption by air or the solvent is strong. In previous work, we investigated single strands of adenines that form a stacked single helical conformation at neutral pH [12–15]. It was found that the bases in single strands containing only adenines[1] (A_n, $n = 2–30$) electronically couple in the excited state in agreement with works by others [16,17], and that the extent of this coupling is state dependent and large in states accessed by VUV (<200 nm) radiation. In the UV range (>200 nm) the coupling was shown to be limited to nearest neighbor interactions, whereas the coupling in the VUV range was shown to extend over eight bases [11]. We later carried out a similar study on single strands of thymine (T_m, $m = 1–5,7,10$) and showed that only nearest-neighbor interactions are present at all wavelengths in the range from 170 nm to 330 nm [10]. To probe the importance of the base sequence we then carried out experiments on samples of the form AT_mA with $m = 1–3$ and found that the SRCD spectra could be reasonably well reproduced from sum spectra containing spectra of AT, TA, T_m and T [18]. The absence of an A_2 adenine coupling term suggests that the introduction of a thymine spacer, even a single one, dramatically reduces the adenine–adenine coupling. However, the amount of data was too limited to draw a firm

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[1] The sugar is deoxyribose and for simplicity throughout this paper we omit the d in the strand sequence, *e.g.* $(dA)_n$ will be written A_n.

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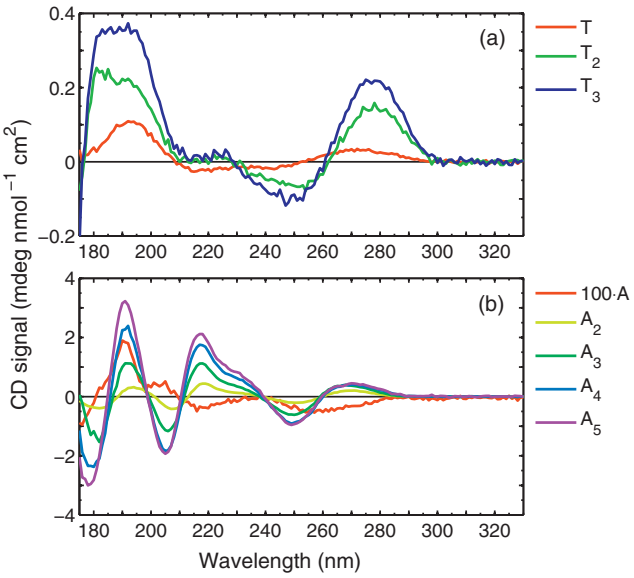

Fig. 1. (a) SRCD spectra of T_m, $m = 1–3$. (b) SRCD spectra of A_n, $n = 1–5$.

and general conclusion on this important issue, also considering the short length of the strands. We therefore decided to carry out a more extensive study in which we examined the influence of thymine spacers on the electronic coupling between adenines in single strands in a more systematic manner. The thymines were placed as a unit in the middle of each strand and the number of adenines and thymines were varied separately. The shortest strand contained only three bases and the longest 13 bases. The results from this work are reported here.

2. Experimental

Synchrotron radiation circular dichroism (SRCD) spectra of oligonucleotides in aqueous solution were measured. The oligonucleotides subjects for study were: $A_nT_mA_n$, with $n = 1–5$ and $m = 1–3$, A_n with $n = 1–5$ and T_m with $m = 1–5$. All oligonucleotides were purchased from DNA Technology A/S, Aarhus, Denmark. Known amounts of the DNA strands were dissolved in pure water and SRCD spectra were obtained at the CD1 beam line at the ASTRID storage ring facility in Aarhus, Denmark [19,20]. At the beginning of each filling of the storage ring the setup was calibrated for wavelength and optical rotation magnitude. Spectra were recorded at 20 °C and measured using a quartz cell type QS124 with a path length of 0.1 mm (Hellma GmbH, Germany). The range of wavelengths measured was between 170 nm and 330 nm. All spectra were averaged, baseline subtracted and slightly smoothed with a Savitzky–Golay filter using the CD data processing software CDTool [21]. Concentrations were determined from the measured absorbance at 260 nm and calculated extinction coefficients at 260 nm [22,23].

3. Results and discussion

To quantify the effect of introducing thymine spacers between two sequences of adenine bases in a single strand, it is useful first to examine the spectra of single strands with identical bases. The spectra of T_m, $m = 1–3$ and A_n, $n = 1–5$, are shown in Fig. 1 [10,11]. For both thymine and adenine the monomer signals are different from those of longer strands, and they are much smaller in intensity (notice the adenine monomer signal is multiplied by a factor of 100 in Fig. 1b), while the signals of the longer strands all exhibit the same spectral features. This clearly illustrates that CD spectroscopy probes the interactions between the bases.

SRCD spectra of $A_3T_mA_3$ are shown as representative examples of $A_nT_mA_n$ in Fig. 2a. The $A_3T_mA_3$ spectra resemble each other, even with respect to the intensities of the bands. The spectra are very similar to the adenine single strand spectra; both the band positions and relative intensities are similar. This shows that the SRCD strand signal does not depend on the number of thymine spacers; either the coupling is completely broken by just one spacer or the adenine bases couple just as well through three spacers as through one. In Fig. 2b the SRCD spectra of $A_nT_2A_n$, $n = 1–5$, are shown. When the spectra of $A_nT_2A_n$ in Fig. 2b are compared to the spectra of adenine and thymine single strands (c.f. Fig. 1), A_{2n} and T_2, it is evident that no new spectral features appear when T-spacers are inserted. The more CD active adenines dominate the spectra.

In the following analysis S denotes a spectrum, *e.g.*, the spectrum of $A_nT_mA_n$ is denoted $S(A_nT_mA_n)$. As mentioned in the introduction, no adenine–adenine coupling term is needed to reproduce the CD signal of AT_mA. This again is evident here since the difference spectra given by $S(AT_mA) - S(T_m)$ do not resemble neither $S(A_2)$ nor 2 $S(A)$, but can be reproduced from the sum spectrum of the couplings between AT and TA given by $S(AT) + S(TA) - 2\,S(T)$, Fig. 3a. A term 2 $S(A)$ should in principle be subtracted from this linear combination but is omitted due to the negligible signal of A. This verifies that the difference spectra of the short strands with only one adenine in each end are due to the two couplings between adenine and thymine: AT and TA.

To determine if decoupling occurs in the longer strands, we calculated the difference spectra $S(A_nT_mA_n) - S(AT_mA)$. Hence the difference spectra are free of adenine – thymine couplings. We then compare the difference spectra with $S(A_{2n})$ and 2 $S(A_n)$, respectively. If all adenines in the strand $A_nT_mA_n$ are allowed to couple, the difference spectrum should resemble $S(A_{2n})$. If no electronic coupling is possible through the T-spacers, the difference spectrum should resemble 2 $S(A_n)$. This comparison is shown in Fig. 3 for all molecules investigated. From Fig. 3b we see that the difference spectra, $S(A_2T_mA_2) - S(AT_mA)$, with $m = 1–3$ are identical, and they are also identical to 2 $S(A_2)$ for all wavelengths. The signals in $S(A_4)$ are larger than those of the difference spectra. This difference increases with decreasing wavelength and is quite high in the VUV. This clearly shows that the electronic coupling between the two adenine ends is broken in the VUV as well as in the UV in the case of $A_2T_mA_2$.

For the remaining investigated molecules it is evident from Fig. 3c–e that it is not possible to determine whether decoupling occurs or not in the UV-region. All spectra are almost identical. This

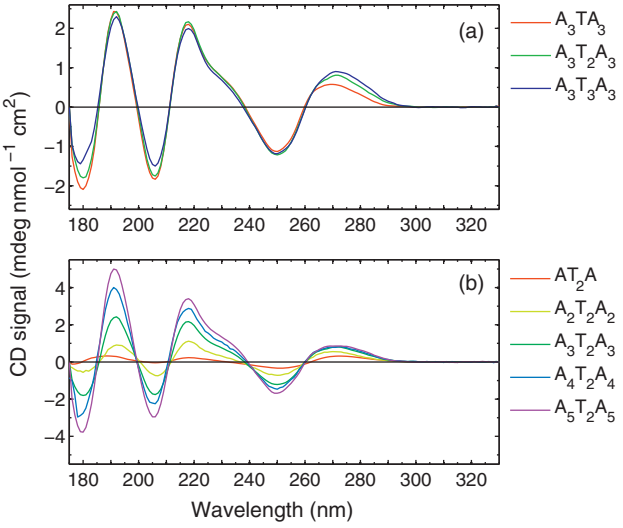

Fig. 2. (a) SRCD spectra of $A_3T_mA_3$, $m = 1–3$. (b) SRCD spectra of $A_nT_2A_n$, $n = 1–5$.

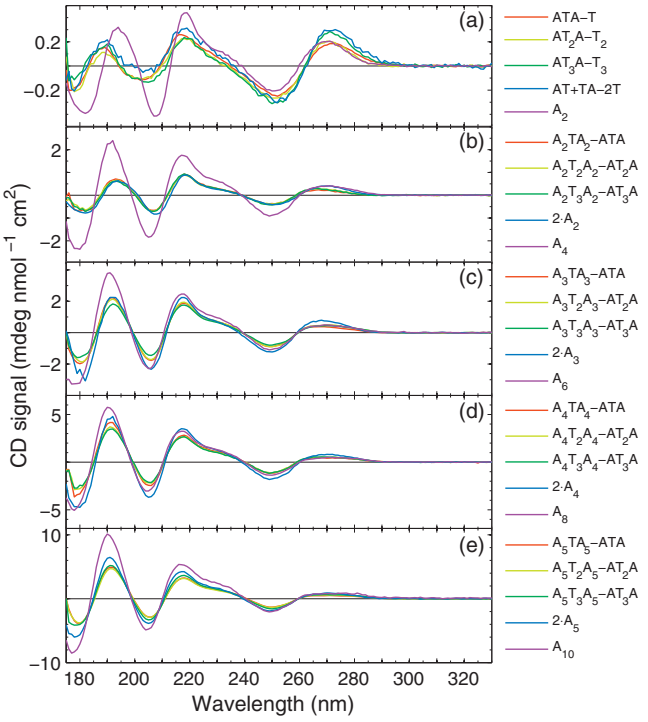

Fig. 3. Difference spectra, $A_nT_mA_n - T_m$, $n = 1–5$ and $m = 1–3$ and the associated spectra of A_{2n} and 2 A_n.

is not surprising since only nearest-neighbor couplings between the adenines are present in the UV-region and the 2 $S(A_n)$ and $S(A_{2n})$ spectra only differ by one adenine–adenine neighbor coupling. A difference of only one nearest-neighbor coupling is small (A_2 spectrum in Fig. 1b) compared to the difference spectra in Fig. 3c–e, and therefore it is not possible to clarify if decoupling occurs. In contrast the VUV-region shows clear differences, and the interpretations of the four longer strand series in Fig. 3b–e are straightforward. The difference spectra are identical to 2 $S(A_n)$ so decoupling is indeed induced by the T-spacers. Whether all possible couplings between the adenines are broken or only a part of them, we cannot tell from these spectra due to the uncertainties in peak amplitudes. However, it is evident that even a single thymine spacer breaks the majority of the couplings between adenines in a single strand.

While oligonucleotides of adenine form stacked single-helical structures at neutral pH [12–15], it is possible that the introduction of thymine spacers produces somewhat different structures. We note that the salt concentration was kept low to prevent duplex formation. Furthermore, the flexibility of a single strand is high, and it may depend significantly on base sequence, which would to a certain degree impact on the electronic coupling between bases [24,25]. However, the similarity of the spectra as revealed from the rigorous mathematical analysis indicates that the structures of the strands on average are all quite similar. A firm establishment of the structures calls for NMR experiments.

4. Conclusions

In conclusion we have shown that the electronic coupling between adenine bases in single-stranded DNA is absent when they are separated by thymine spacers. In the UV region where only nearest neighbor couplings are present between adenines in a sequence, it is not surprising that all electronic coupling is broken by simply incorporating a spacer, also considering that the structure and the flexibility of the strand may have changed. However, in the VUV where as much as eight bases in a sequence couple, it is unexpected that one thymine spacer is enough to break the interaction and form islands of adenines. This allows for a control of the electronic coupling between bases that may be of importance for the construction of DNA nanodevices.

Acknowledgements

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