Short Oligonucleotides Aligned in Stretched Humid Matrix – Secondary DNA structure in Poly(Vinyl Alcohol) Environment

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Abstract

We report that short synthetic, double as well as single stranded DNA, can be aligned in stretched humid poly(vinyl alcohol) (PVA) matrix and the secondary structure (nucleobase orientation) can be characterized with linear dichroism (LD) spectroscopy. Oligonucleotides of lengths varying between 10 (3.4 nm) and 60 bases (20.4 nm) were investigated with respect to structural properties in the gel-like polymer environment. The DNA conformation as a function of relative humidity reveals a strong dependence of helical structure of DNA on PVA hydration level, results of relevance for nanotechnical studies of DNA-based supramolecular systems. Also, the PVA gel could provide possibilities to test models for nucleic acids interactions and distribution in cell contexts, including structural stability of genetic material in the cell and PVA-packaging for gene delivery. A method by which duplex oligonucleotides, with sequences designed to provide specific binding sites, become amenable to polarized-light spectroscopy opens up new possibilities for studying structure in DNA complexes with small adduct molecules as well as proteins.

Key words: DNA nanotechnology, PVA-DNA, PVA-oligonucleotides, reduced water activity, anisotropic polymer films, linear dichroism spectroscopy, LD

Introduction

Nanobiotechnology has become one of the fastest developing areas within biosciences where understanding the interactions between bio-macromolecules on a molecular level is a challenge. Short synthetic DNA oligomers may also be used as building blocks in two- and three-dimensional nanostructures[1-4], or more complicated constructs on a larger scale be assembled utilizing origami technique[5-7]. While oligonucleotides coupled to nanoparticles or quantum dots[8-11] provide interesting implementations of nanotechnology into the fields of biology and medicine, there is a general lack of knowledge how such constructs might be affected by change of external conditions such as water content and presence of hydrophobic polymer constituents. More specifically, understanding how water activity influences DNA structure is of fundamental importance for applications of nanobiotechnology in which individual DNA strands act as structural elements, as well as for fundamental understanding of molecular interactions at gene level in the nucleus and in spores at low water content.

One approach taken in various contexts is to form a composite of DNA incorporated in a matrix of poly(vinyl alcohol) (PVA), a water soluble synthetic polymer which is known as an excellent host matrix for hydrophilic molecules such as DNA when having a large water content[12-15]. Water content in humidified films of PVA can be as high as 50% w/w [16] and can be easily controlled by adjusting relative humidity conditions in a closed chamber in which the mounted film is kept. Previous studies in PVA have been restricted to long DNA, the shortest being sonicated fragments of lengths around the DNA persistence length (50 nm). A

few early attempts in our laboratory to study very short DNA (10 bp) failed, possibly due to suboptimal, denaturing solution conditions and less sensitive measuring technique.

Here we report that indeed also short oligonucleotides can be aligned in double-stranded form in stretched humid poly(vinyl alcohol) (PVA) matrix but that complete destacking and denaturation occurs when water activity is below a critical threshold. The dense gel environment, which might also be thought of as mimicking molecular crowding in the cell nucleus, is excellent for investigating how structural properties of oligonucleotides are affected by confinement and lowered water content. Also, it is a relevant system to study in context of using polymer gels as delivery platforms in genetic and drug delivery applications, as short DNA and duplex RNA (siRNA) are extensively exploited in genetics as a tool for targeting DNA (antigene strategy) [17] or RNA (antisense strategy) [18, 19]. For the latter applications usually, the nucleic acids are in mixtures with non-toxic polymers such as polyethylenimine (PEI) [20] , polyethylenglycol (PEG) [21] or poly(vinyl alcohol) (PVA) [14, 22] that may constitute protection shells for the delivery process. Generally little is known about the influence of the polymers on properties and conformation of the nucleic acids[23].

Using linear dichroism spectroscopy we have investigated properties of short synthetic DNA incorporated in PVA as a function of relative humidity and oligonucleotide length.

Materials and Methods

Materials. Poly(vinyl alcohol), PVA, with an average molecular weigth of 80,000 was purchased from Du Pont (Sweden) under the commercial name Elvanol 71-30. Oligonucleotides were obtained from ATDBio (England) and used without further purification. In order to form a duplex, equimolar concentrations of complementary strands were mixed followed by hybridization. First, the sample was heated to 85 ^{o}C and then subject to stepwise cooling to 20 ^{o}C was done.

Tab. 1 Oligonucleotides sequence composition and base concentration*

base	sequence	GC content	Stock conc. per single strand (μM)
10 mer	Seq. 1: 5'-CATCTGTTCG-3' Seq.2 5'-TCTGAACCAC-3'	50%	322
14 mer	5'-TCCGTCTGCAGCGT-3'	64%	276
20 mer	5'-ATGGCATCAGGCAGTTTAAC-3'	45%	45,5
40 mer	5'-ATGGCATCAGGCAGTTTAACATGGCATCAGGCAGTTTAAC-3'	45%	35,4
60 mer	5'-ATGGCATCAGGCAGTTTAACATGGCATCAGGCAGTTTAAC ATGGCATCAGGCAGTTTAAC-3'	45%	29,7

*Oligonucleotides dissolved in 2 mM NaCl buffer prior mixing with PVA.

Film sample preparation. PVA powder was dissolved to 10% (w/v) in a 2 mM NaCl solution by first mixing it during stirring for 5 min at room temperature. This PVA mixture was then incubated at 80 ^{o}C (water bath) for 30 min while stirring, until a transparent viscous solution was obtained. Samples containing PVA and DNA were prepared by mixing the two constituents at volume ratios 100:10 using stock solutions with the following concentrations: 10% w/v (0,1g/ml) PVA and DNA oligonucleotide see Table 1.

PVA films containing oligonucleotide were prepared following established procedures for PVA-DNA films[24]. Briefly, the PVA-DNA solution (typically 1 ml) was poured on to a glass slide and allowed to form a dehydrated PVA sheet (2 days at room temperature in dust-free environment). The sheets were then assembled into a stretching device and hydrated to

become humid films, inside a closed chamber (with silica windows allowing optical measurements) where relative humidity was controlled by the equilibrium water pressure above a salt solution in a container at the bottom of the chamber (Table 2). The dry PVA sheet, which is brittle, takes up water rather quickly (30 minutes) to become rubber-elastic and easy to stretch without risk of rupture.

Table 2 Salt buffers used for gel films equilibration

Saturated aqueous buffer solution at bottom of chamber	Relative humidity (r.h.) in closed chamber containing film sample
NaCl	75%
KBr	80%
Na_2CO_3	90%
Na_2SO_4	93%
H_2O	100%

Finally the hydrated films were stretched under the same controlled humidity conditions to a predetermined stretching ratio $R_s = D_{\parallel}/D_{\perp}$ between length and width of stretched film (in this study R_s=1.5 if not otherwise stated). The time-dependence of DNA behaviour in humid films was studied in two ways: one approach was repeated spectral measurements on a stretched film (after it was humidified for 30 min), a second approach was to equilibrate unstretched films in different relative humidities and for each record spectra directly after stretching.

UV-Vis Spectroscopy. Absorption spectra were recorded on a CARY-5000 spectrophotometer, using as a reference PVA films prepared as above but lacking the DNA.

Linear Dichroism. Linear dichroism (LD) is defined as the difference in absorbance of light linearly polarized parallel ($A_{\parallel}$) and perpendicular ($A_{\perp}$) to the macroscopic axis of orientation (in this study the stretching direction of the film).

$$LD = A_{\parallel} - A_{\perp} \quad (1)$$

LD spectra of short DNA molecules oriented in such stretched films can provide information on the rate of orientation and structure of DNA while a non-stretched (isotropic) film should exhibit zero LD. LD spectra were recorded on a Chirascan CD spectrophotometer (Applied Photophysics) equipped with an LD accessory unit – a stretching device with the film sample inside a humidity chamber.

The reduced LD (LD^r) is obtained by dividing the LD by the absorbance of the corresponding isotropic sample (A_{iso}), which for an anisotropic uniaxial sample can be obtained as $A_{iso} = (A_{\parallel} + 2A_{\perp})/3$, [24] so that

$$LD^r = \frac{LD}{A_{iso}} = \frac{3 \cdot LD}{A_{\parallel} + 2A_{\perp}} \quad (2)$$

For molecules with a uniaxial orientation distribution, as can be expected for DNA in PVA matrix since the films are thick (30 µm for dry film) compared to the helix diameter (2 nm), LD^r is a product of an orientation factor S and an optical factor O.

$$LD^r = S \cdot O = \frac{3}{2} S(3\cos^2\alpha - 1) \quad (3)$$

The optical factor O is related to the angle α between the local helix axis and the light absorbing transition moment of a DNA base. The orientation factor $S = \frac{1}{2}(3\langle\cos^2\theta\rangle - 1)$ corresponds to the effective orientation of the DNA helix in the PVA film, where θ is the

angle between the macroscopic stretching direction and the local helix axis of a particular molecule, and where the average runs over all parts of DNA molecules in the sample. S=1 for perfect orientation of helix axis parallel with stretch direction, i.e. Θ =0. The degree of helix orientation *S* was calculated from the LD^r values at 260 nm using the average angle α_{DNA} =86^o for the DNA bases [25]. For the small stretch ratio R_s = 1.5 (corresponding to 50 % stretch of film) the orientation is generally poor: S= 0.011, for the 20-mer ds-DNA at 100% r.h., while for 300% stretch the same oligonucleotide showed S=0.1, i.e. similar to the degree of orientation that is frequently observed in flow LD of very long DNA.

Electrophoresis. Double and single stranded 40- and 60-mer oligonucleotides together with bromophenol indicator dye (Fermenta) were run on a 4% methapor gel in 0.5x TBE buffer for 60 min at 120 V, 40 mA. The gel was then stained with ethidium bromide, left for 30 minutes and scanned using a Typhoon 9401 gel scanner with excitation at 488 nm and a 40 nm emission band pass filter centered at 520 nm.

Melting experiments. Duplex DNA was formed by mixing equimolar concentrations of complementary single-strand oligonucleotides adjusting concentration to about 1 AU prior to melting experiment. Samples of 10, 14, 20, 40 and 60-mers were put in melting cells in the Cary 4000 absorption spectrophotometer and left overnight for two cycles of melting, starting at 15 ^{o}C, then stepwise (1°C per step every 5 min) heating to 85 ^{o}C and back to 15 ^{o}C.

Results

A series of single (ss) and double stranded (ds) oligonucleotides of lengths ranging between 10 and 60 base-pairs/bases were investigated in stretched poly(vinyl alcohol) gel equilibrated

at 75% r.h. or 100% r.h. Figure 1 presents an overview of LD signs and shapes indicative of the DNA conformation at the various conditions.

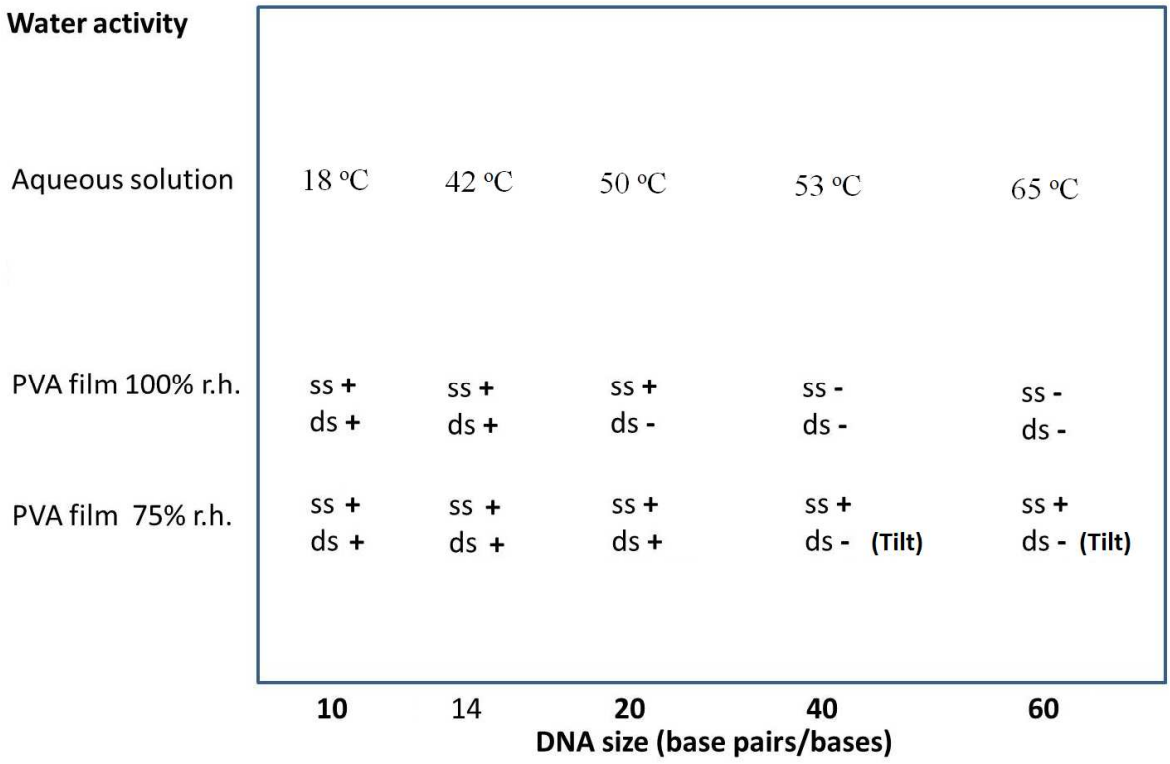
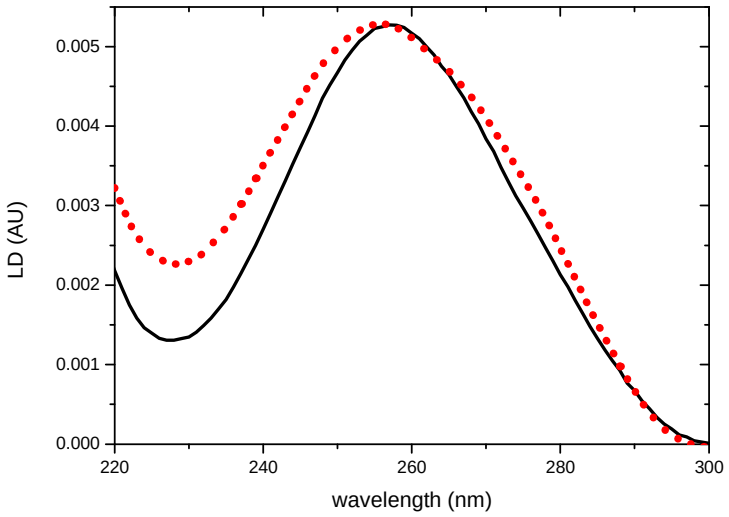


Fig. 1 Overview of LD results (LD sign: + or - , and indication of base tilt) of single-stranded (ss) and double-stranded (ss) oligonucleotides in stretched humid PVA matrix containing 2mM NaCl, at 20 °C and at relative humidity 100% or 75%. Melting temperatures in 2mM NaCl aqueous solution also indicated.

The linear dichroism (LD) of the 10-mer oligonucleotide in 2mM NaCl is positive for both dsDNA and ssDNA with a maximum at 258 nm and 256 nm, respectively, which minor wavelength difference appears not to be due to sequence variation but most likely to different interactions of ss- and ds-DNA with PVA, for example, PVA forming hydrogen bonds with unpaired nucleobases [Fig. 2A, sequence composition see Table 1]. The LD results indicate that each nucleotide is oriented independently in line with the polymer chains, more or less like the orientation observed in PVA samples containing pure nucleobases[26]. This observation is in good agreement with melting experiments that show denaturation of 10-mer DNA in

2mM NaCl already in room temperature in water solution, before mixing with PVA [Fig. S1 A]. Similar positive LD spectra were also obtained for the 14-mer oligonucleotide indicating destacked nucleobases and again with distinct difference in absorption maxima for ssDNA and dsDNA, at 263 nm and 256 nm, respectively [Fig. 2B]. These results together with melting experiments indicate that 14-mer DNA in 2 mM NaCl forms a stable duplex in solution [Fig. S1 B] but becomes immediately destacked when incorporated in PVA film.

A



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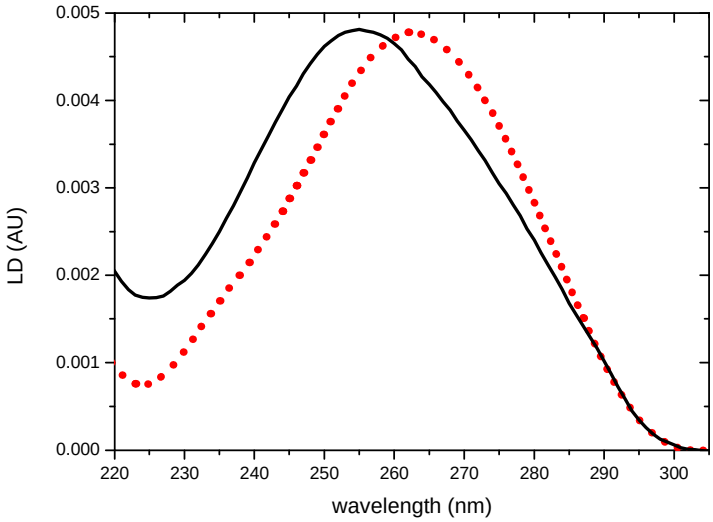

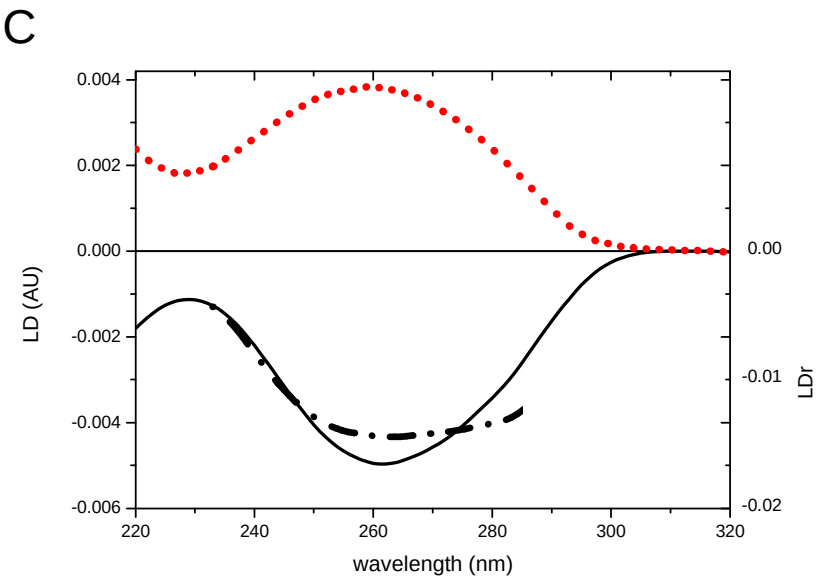

Fig 2. Linear Dichroism (and reduced Linear Dichroism, LD^r) spectra of single-stranded (ss) and double-stranded (ds) DNA in stretched humid PVA matrix at 100% r.h. and containing ca 4 mM NaCl. **A**. 10 bases/base-pairs long ss (dotted) sequence 1 and ds (solid) oligonucleotide Sequence 2 . **B**. 14 bases/base-pairs ss (dotted) and ds (solid). **C**. 20 bases/base-pairs ss (dotted) and ds (solid) and LD^r (calculated from Eq (2), dashed). Film samples equilibrated for 4 h and subsequently stretched to ratio R_s = 1.5. For all spectra PVA baseline was subtracted and LD intensity normalized to the same peak height for the sake of comparison. Sequences given in Table 1.

The LD spectrum is negative for a 20 bp DNA duplex, while it is positive for a 20 bases long single stranded DNA of the same sequence [Fig. 2C]. For longer oligonucleotides, such as double-stranded 40 bp oligonucleotides and single-stranded 40 bases DNA, both ss and ds forms display negative LD spectra in 100% r.h. [Fig. 3A, S3]. Similar results were obtained also for 60-mer double- and single stranded DNA [Fig. 3B , S4] under the same relative humidity conditions as for the 40 mer. Melting experiments and electrophoresis [Fig S1 and S2] confirm that both duplex and single strand structures of 40- and 60-mer DNA are stable in solution.

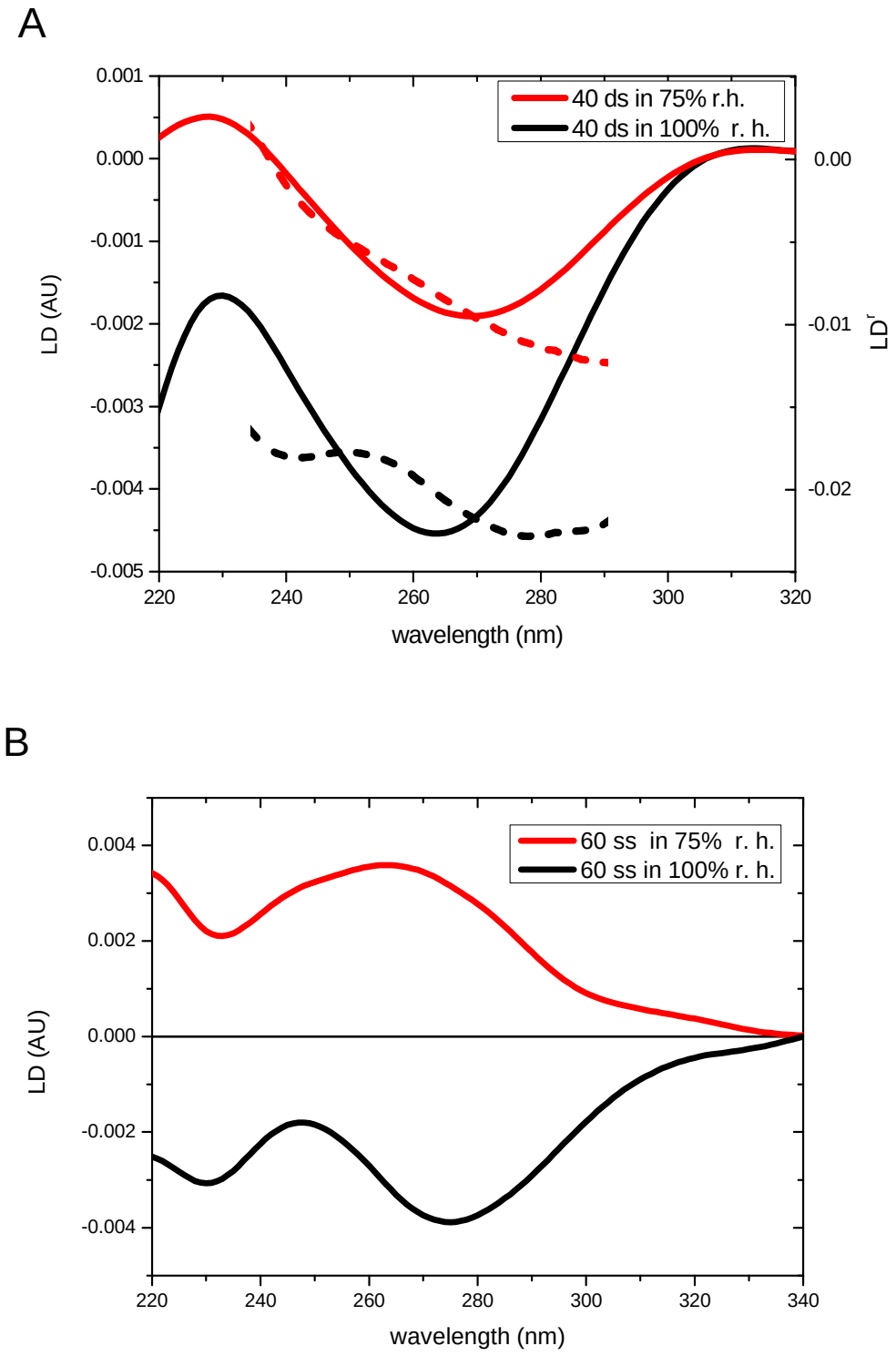

Fig. 3. **A** LD spectra of 40-mer ds DNA in PVA-films at indicated relative humidities (r.h.) and containing ca 4mM NaCl: 75% r.h. (red solid) and 100% r.h. (black solid) and LD^r from Eq. 2 (dashed). **B**. LD of single stranded 60-mer in 100% r.h. (red solid) and in 75% r.h. (black solid) PVA. All film samples were equilibrated in chamber with relative humidity adjusted to 75% r.h. or 100% r.h. for at least 4 h prior the measurement.

Experiments were performed with DNA oligomers in PVA films at reduced relative humidities to study the effects of dehydration. For the double-stranded 20-mer, the negative LD spectrum changes from negative to positive when the water content is below 93% r.h., but the change is reversible and the negative spectrum recovers after equilibration at high r.h. [Fig. 4A]. Thus, a few days of equilibration at 75%, 80% and 90% r.h. all result in a positive LD band with maximum around 260 nm and a distinct shoulder at 275 nm - suggesting that the nucleotides are no longer perpendicular to the DNA helix axis but tilted in the PVA. However, when the humidity in the closed chamber was readjusted to 93% r.h., the nucleotides were found to reorient themselves and the LD spectrum return from positive to negative shape within a few hours [insert B in Fig. 4A] indicating that in presence of water the hydrophobic stacking forces make the bases ”renature” into a structure in which their base planes are preferentially perpendicular to the fiber direction. Since the film is rather rapidly humidified (<30 min) most of the kinetics can be assigned to rearrangement of DNA conformation.

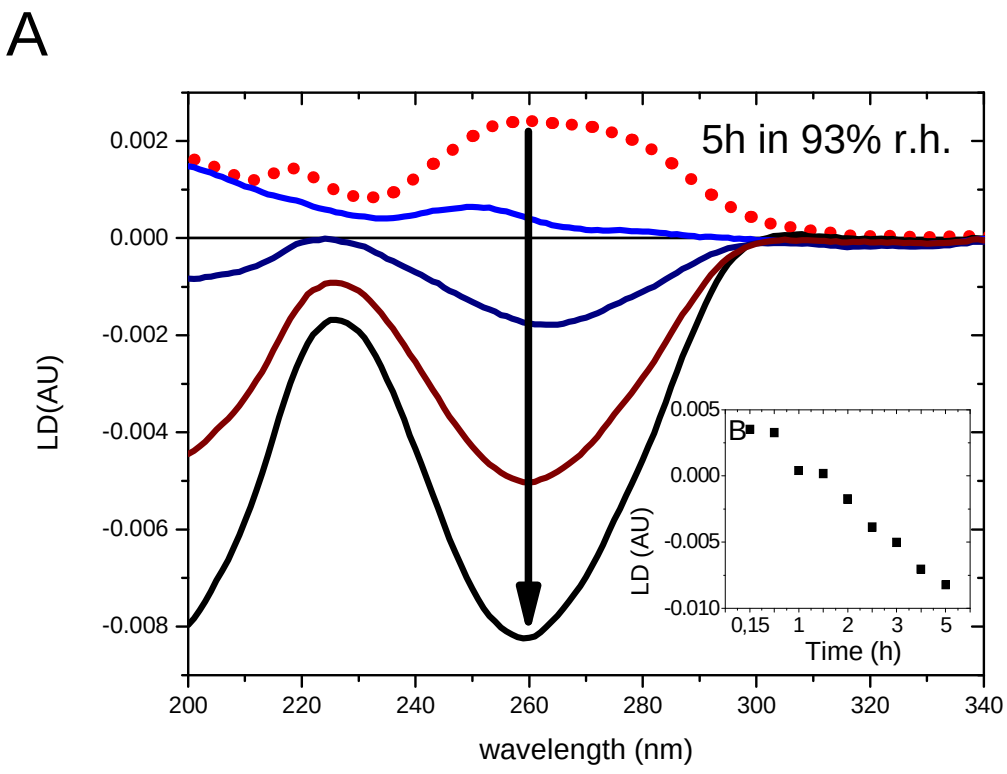

Fig 4. **A**. LD of PVA film containing 20bp ds DNA showing positive LD when equilibrated at 90% r.h. (dotted) but negative LD when relative humidity readjusted to 93% r.h. (solid spectra recorded after 15, 30,120 and 300 minutes after raising the humidity in the sample chanber) indicating a renatured double-helix DNA structure. **B**. Kinetics of the DNA B form reformation during 5 hours. For all LD spectra and intensities a baseline for a PVA reference without DNA was subtracted.

The longer 40 and 60 mer single- and double-stranded DNAs were studied in order to see whether oligonucleotide length is an important factor for conserving the helical structure in dehydrated environment. Negative LD spectra were recorded for 40 bp (Fig. 3) and 60 bp (Fig. 3A, 4S) oligonucleotides equilibrated in 75% r.h. exhibiting a distinct LD maximum at 262 and 258 nm, respectively. The shape of the LD spectrum for the 40-mer at 100% r.h. is similar to the absorption spectrum (rather constant LD^r) suggesting a B like type of conformation with the nucleobases preferentially perpendicular to the helix axis, while at 75% r.h. a variation in LD^r with wavelength consistent with A form is observed (cf Fig 6 in Ref. 26).

The negative peaks for double-stranded DNA are significantly suppressed at 75% r.h. compared to at 100% r.h. indicating also a poorer orientation in addition to the conformational change from the high r.h. condition. An important message here is that the helical structure appears to be retained in a dehydrated environment in case of longer oligonucleotides. However, dehydration clearly affects the structures of single stranded oligomers aligned in PVA quite differently (Fig. 3 B): while ss 60-mer shows negative LD at 100% r.h. and, thus, base stacking perpendicular to the orientation direction, at 75% r.h. LD is positive indicating that the bases are then unstacked and oriented more parallel with the stretch direction.

In order to exclude higher order structures due to accidental self-complementary base pairing, electrophoresis was applied, indeed confirming no significant presence of such structures [Fig 2S]. In conclusion, the LD spectra of the single stranded 40-mer and 60-mer DNA both change signs from negative at 100% r.h. to positive when the relative humidity is decreased to 75% due to secondary structural changes induced by the presence of the PVA and the lower water activity [Fig. 3B, S4].

In order to investigate how fast hydrophobic interaction with PVA may influence the structural properties of the DNA helix, repeated absorption experiments were performed. Both single stranded and double stranded 20-mers were first equilibrated at 100% r.h. and then left drying at room conditions in an absorption cell on which repeated scanning of the samples was performed at 1 minute intervals. A marked increase of the single-strand 20-mer absorption confirms that the hydrophobic interactions with the PVA in the drying state takes over and decouples the π-stacking interactions between adjacent bases, leading to significant hyperchromicity (Fig. 5). For the duplex form the change in absorption is markedly stronger than for the single-strand DNA, as expected if the ds-DNA is melting. However, the percentage of hyperchromic effect in PVA is still relatively small compared to DNA melting

in water solution. This is possibly so because the nucleotides remain in close proximity with each other confined by the highly viscous surrounding PVA matrix even when DNA melts.

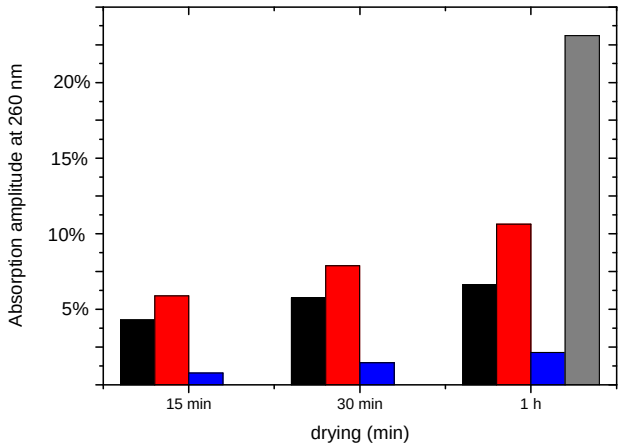

Fig. 5. Absorbance change of single-stranded (black) and double-stranded (red) 20mer oligonucleotide in PVA first equilibrated overnight at 100% r. h. conditions then left drying at room temperature. Melting hyperchromicity shown for 20 bp oligonucleotide (grey). Drying of pure PVA (blue) shown for comparison. Absorbance spectra converted to % scale of change for sake of comparison.

Discussion

Structural properties of short oligonucleotides in PVA

Positive LD has previously been reported for single DNA bases[26] as well as for long DNA[12-15] in stretched dry PVA films. Our results presented here for the shorter oligomers could be seen as a direct generalization in terms of base orientation. The results clearly show that each nucleobase in short DNA strands, in analogy to their single counterparts, is oriented with their plane in line with the stretched polymer chains. For planar small aromatic molecules in anisotropic polymer matrices one observes rod-like orientation for very elongated molecules but more disk-like orientation with less elongated molecules like the nucleobases[27]. Also the bond between nucleobase and sugar may influence the way a nucleoside will be aligned: if the bond is preferentially directed perpendicular to the

macroscopic orientation axis, transitions parallel to this direction will appear with negative LD (and transitions perpendicular to the bond with either positive or negative LD depending on the preferred orientation of the plane), whereas if the bond points more along the orientation axis all transitions along the bond will give positive LD and transitions perpendicular to it will have negative LD[12-15]. The observed solely positive LD indicates that the orientation is determined mainly by the disk orientation mechanism and thus that the supporting bonds are less influential.

The limiting case is the 20 bp double strand, which is the shortest oligonucleotide that exhibits a negative LD and also the shortest DNA for which LD has been reported. Its constant LD^r over the region 230-285 nm [Fig. 2C], and curve shape similar to the absorbance curve, are features identical to those of the canonical B-fibril conformation reported previously for long as well as for sonicated DNA in polymer films in 100% r. h.[24], in which the DNA strand is oriented in the stretch direction, and the bases are stacked preferentially perpendicular to the helix axis. By contrast, the single strand 20-mer oligonucleotide, as well as the single and double stranded shorter oligonucleotides, obviously lack the same level of internal interactions necessary to stabilize a helical structure so that it can become oriented in the PVA matrix.

One possible reason for the structural disruption of the shorter strands is reflected by their low melting temperature, probably contributed to by a lower effective relative dielectric permittivity of PVA (28) compared to water (78) which affects intermolecular as well as intra-helical interactions of nucleic acids. As a consequence of decreased π-stacking between the bases, which normally provides the hydrophobic environment that is a prerequisite for strong inter-base hydrogen bonds, the hydrogen bonds between the bases are breaking up in PVA and the DNA structure is collapsing. The increased GC content of the 14-mer which in aqueous solvent stabilizes the structure compared to the 10-mer, apparently does not

sufficiently improve the stability of short oligomer and its lack of helical structure is consistent with the positive LD that is observed for all DNAs shorter than 20 bp [Fig 2C]. We conclude that sequence composition is not of significant importance for whether the secondary structure of short oligonucleotides will be retained in the polymer environment.

A central result of this study is the finding that a relatively short (20 bp) oligonucleotide DNA may be aligned and studied by LD in humid PVA, since this could open up new structural applications for the LD technique to various complexes to specific DNA sequences, where so far only long non-specific DNA have been amenable to study[27]. The negative LD at 260 nm and band shape over the region 235-285 nm, similar to the absorption band i.e. having approximately constant LD^r in this wavelength region, is a strong indication for B-form conformation of DNA previously only reported for long and sonicated DNA in 100% r.h. PVA [12, 24, 25]. The calculated S factor for high stretching ratios (R_s = 3.0) of PVA is as high as 0.1 for the double stranded 20-mer, thus in the vicinity of what is normally observed for very long DNA free in aqueous solution in Couette flow[27]. However, in the case of the single stranded 20 bases of the same sequence as in the duplex a positive LD is instead observed. This indicates that without the complementary strand the bases instead prefer to form hydrogen bonds or stack with the PVA which surrounding matrix also provides steric confinement. For long DNA positive LD has also been observed previously in dry PVA stretched after heating, but with LD maximum considerably more red-shifted suggesting that the backbone affects the angular distribution of the nucleobases giving preference to transitions polarized parallel to the base-sugar bond[12]. In conclusion, the bases of DNA appear to be vulnerable to dielectric variations of the environment in the humid polymer gel and also depend sensitively on the accumulated or cooperative effects of base stacking, i.e. on the length of the oligonucleotide. This is the reason why we also investigated the 40-mer and 60-mer, single-stranded as well as double-stranded DNA oligonucleotides, for which good

alignment and structural stability is observed at 100% r.h. (Fig 3 and Fig S3 and S4) further confirming the importance of stacking interactions in the double helix.

To sum up, the positive LD of the single stranded 20-mer, compared to its double stranded counterpart, clearly confirms the Watson-Crick base-pair hydrogen bonds being the key factor to stabilize the double helix. Without complementary nucleotides, since the base-base stacking is cooperative and therefore relatively weak in short oligonucleotides, a stacked structure is not adopted by a 20-mer single strand. In conclusion, the base-base interactions in a helix are vulnerable to the dielectric environment of humid polymer films and depend on oligonucleotide length.

Water dependent secondary structure of 20 bp DNA in PVA

Alignment of 20 bp oligonucleotide in PVA gives an opportunity to investigate structural properties of short synthetic DNA in dehydrated conditions using linear dichroism. The sign flip behavior in the LD spectrum of the double stranded 20-mer in reduced humidity shows how the amount of water molecules directly influences the secondary structure of the B-form duplex. In the range from 75% to 90% r. h., A-form DNA has been reported for long DNA in PVA[24], dehydrated films[28] and fibres[29]. Lack of A conformation for the 20 bp oligonucleotide, especially above 80% r.h., where the primary hydration shell around the helix is expected to be fully formed[30, 31], is thus intriguing and shows, again, that the cooperative base-base stacking over a minimum number of bases is a prerequisite for a defined secondary structure, whether B-like or A-like. On the one hand 25 water molecules per nucleotide pair including 17 bound tightly[28] forms a separation layer from polymer environment but on the other hand the PVA matrix may enthalpically (hydrogen bonds to OH groups) assisted by entropy of dissociation wrench off some water molecules that are bound less strongly to DNA. Also in addition, interactions with the hydrophobic crystalline regions of the polymer can influence

the DNA properties and lead to nucleotide separation as is observed in case of oligonucleotides shorter than 20 bp.

When humidity in the closed chamber is adjusted to 93% r.h., a slow (hours) reorientation of nucleotides consistent with the change of LD sign from positive to negative is seen [Fig. 4]. Catalyzing factor for the rearrangement is the amount of water molecules present in the system. For pure humid DNA fibers, according to Rupprecht, there are 30 ±3 water molecules per base pair in 93-95% r.h. range for B-DNA in its primary hydration shell[32]. These results together with our present observations indicate that increase by 4-6 water molecules per base pair from 25±2 at 80% r.h. to 30±3 at 93% r.h. is a driving force of complete reformation of the protection layer around DNA that allows for further re-hybridization. That is to say that the water structure may be relocated in the system, because the polar, and negatively charged sugar-phosphate backbone that attracts electrostatically Na^+ ions, starts forming a secondary hydration shell which in presence of ca 4 mM NaCl contains 7 water molecules per nucleotide pair[32]. In total there are 37±3 water molecules in both shells per base-pair at 93% r.h. We conclude this to be the smallest amount of water molecules needed to retain the secondary structure of short DNA in PVA.

Another important aspect is the rate by which the effects of hydrophobic interactions caused by PVA appear. The interactions becoming stronger when less water molecules are present in a matrix and increased effects of intermolecular, as contrast to intramolecular, hydrophobic forces in dehydrated films as shown by change of absorption spectra during film drying [Fig.5], can be a reason why no clear A-DNA is observed in PVA but only a direct transition from B-like to molten form below 93% r.h. It is very probable that dehydration may lead to phosphate group reorientation relative to the helix axis, with as much as 25^o between B and A conformation[33]. Then the balance in hydration shells could be disturbed and nucleotides, especially as seen with short DNA, which are more sensitive to hydrophobic interactions with

PVA, may start direct interactions with polymer. The water molecules in the secondary shell are wrenched off and close up in PVA clusters. In conclusion, the transition from B-like form to molten oligonucleotides can be induced directly by PVA if there is not enough water. In this way the change of environment from water to the less polar but still somewhat hydrophilic PVA influences the structural properties and stability of the DNA helix.

40 and 60 mer oligonucleotides in PVA

The pronounced still approximately perpendicular arrangement of the nucleotides in PVA at 75% r.h., indicated by the negative sign in LD, suggests that a helical stacked structure is adopted also in the dehydrated polymer environment even though the primary hydration shell is not complete at that relative humidty[30, 32]. In order to get insight into the actual conformation of DNA, LD^r has been determined for the 40bp duplex [Fig. 3A]. It shows essentially the same wavelength dependence as the theoretical predictions according to the oriented gas model for A-form DNA in PVA, with nucleobases tilted some 20° [24 25]. This indicates that DNA may respond to dehydrated conditions by forming stable A-DNA, but that intramolecular interactions have to be at least equal to or stronger than the dispersion forces of PVA. It seems then, that the sum of π stacking interactions and hydrogen bond formation between bases creates a net stabilizing energy within the double helix of 40bp oligonucleotide but not for the one with 20 bp. Thus, we conclude that strength of intramolecular forces increase with oligonucleotide length. Then the question is if hydrogen-bond interactions and π stacking are equally important for the helix stability. In order to get further insight into this matter, and to understand the role of stacking interactions where no hydrogen bonds between DNA bases are present, a single-stranded 60-mer was also aligned and studied in PVA.

A positive LD was recorded for the 60 bases ss-DNA in 75% r.h. which spectrum can be simply explained by the tilting of bases sitting on branches to the backbone like leaves on a

tree, the destacking being due to dehydration[33]. By contrast, a negative LD was observed at 100% r.h. showing that a pile of bases created by the π stacking can restrain the helical structure in a single strand sequence even without a complementary strand, if there is provided enough water around to contribute significant hydrophobic stacking effect. There is possibly also some interaction due to hydrogen bond formation with polymer hydroxyl groups as suggested by the shift to 275 nm in the LD spectrum at 100 % r.h. [Fig. 3B].

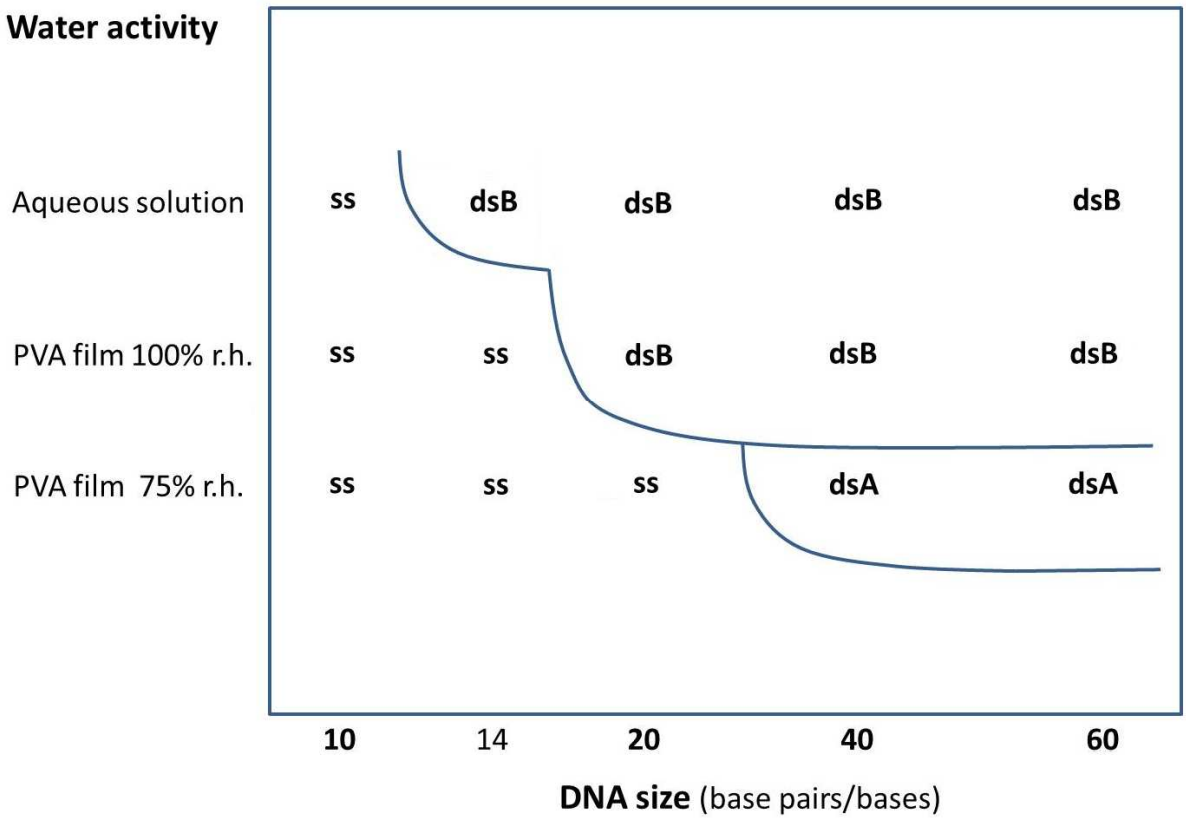

Fig. 6. Phase diagram illustrating how DNA conformation in the PVA environment depends on water activity and oligonucleotide length. Oligonucleotides at 20 ^{o}C in 4mM NaCl.

Conclusions

We are first to report that it is possible to align synthetic DNA oligomers in a stretch-deformed hydrated polymer matrix of PVA and to study their structural properties using linear

dichroism. We find that the secondary structure of the oligonucleotides is strongly dependent on both DNA length and conditions of surrounding environment, schematically shown by the "phase diagram" in Fig. 6. Low water activity causes disruption of short-sequence double helices, while a high water content keeps bases in close proximity so the structure can be reformed when approximately 40 water molecules per nucleotide pair are present. Our results indicate that cooperative stacking interactions play a significant role and longer oligonucleotides can be exposed to lower humidity and still retain their stacked secondary structure even in case of single stranded oligonucleotides. This observation could be important in context of achieving transfection with small DNA or RNA sequences incorporated with synthetic biopolymers, having to enter cells through cell membranes. Anisotropic PVA containing oriented short oligonucleotides may also be a model system for mimicking crowding and realignment in dense cellular systems.

Finally, and from practical point most importantly, our establishment of a system by which duplex oligonucleotides become amenable to polarized-light spectroscopy study, opens up new possibilities for studying structure of aligned DNA samples in complex with small (drug[23]) as well as large adduct molecules (proteins), with sequences designed to provide specific binding sites, a methodology that has until now been limited by the need for very long, and thus unspecific, DNA molecules that could be aligned by flow or electric fields[27].

Supporting information

1. Melting curves of 10 bp, 14 bp, 20 bp, 40 bp and 60 bp DNA, 2. Gel electrophoresis and intensity profiles of 40- and 60 single and double stranded DNA in 2mM NaCl 3. LD of 60 bp in 75% r.h. and 100% r.h., 4. LD of single stranded 40-mer in 75% r.h. and 100% r.h. 5.

Absorption and LD spectra of pure PVA films. This material is available free of charge via the Internet at http://pubs.acs.org.

Acknowledgements

We thank Bobo Feng for discussions and useful comments on the manuscript. The research was supported by grants from the King Abdullah University of Science and Technology (KAUST) and the European Research Council (ERC) to BN.

References

1. Seeman N., Nanomaterials Based on DNA. *Annu. Rev. Biochem.* **2010,** 79, 65-87.
2. Tumpane J.; Kumar R.; Lundberg E. P.; Sandin P.; Gale N.; Nandhakumar I. S.; Albinsson B.; Lincoln P.; Wilhelmsson L. M.; Brown T.; Norden B., Triplex Addressability as a Basis for Functional DNA Nanostructures. *Nanolett.* **2007,** 7, (12), 3832-3839.
3. Andersen E; Dong M; Nielsen M. M; Jahn K; Subramani R; Mamdouh W; Golas M; Sander B; Stark H; Oliveira C. L. P; Pedersen J. S; Birkedal V; Besenbacher F; Gothelf K. V; Kjems J., Self-assembly of a nanoscale DNA box with a controllable lid. *Nat. Lett.* **2009,** 459, 73-76.
4. Gambinossi F.; Banchelli M.; Durand A.; Berti Debora.; Brown T.; Caminati G.; Baglioni P., Modulation of Density and Orientation of Amphiphilic DNA Anchored to Phospholipid Membranes. I. Supported Lipid Bilayers. *J. Phys. Chem. B* **2010,** 114, (21), 7338-7347.
5. Rothemund P. W. K., Folding DNA to create nanoscale shapes and patterns. *Nat.* **2006,** 440, 297-302.
6. Shih M. W.; Quispe J. D.; Joyce G. F., A 1.7-kilobase single-stranded DNA that folds into a nanoscale octahedron. *Nat. Lett.* **2004,** 427, 618-621.
7. Voigt N. V.; Tørring T.; Rotaru A.; Jacobsen M. F.; Ravnsbæk J. B.; R., S.; Mamdouh W.; Kjems J.; Mokhir A.; Besenbacher F.; Gothelf K. V., Single-molecule chemical reactions onDNA origami. *Nat. Nanotech.* **2010,** 5, 200-203.
8. Mirkin C. A.; Letsinger R. L.; Mucic R. C.; Storhoff J. J., A DNA-based method for rationally assembling nanoparticles into macroscopic materials. *Nat.* **1996,** 382, 607 - 609.
9. Fu A.; Micheel Ch. M.; Cha J.; Chang H.; Yang H.; Alivisatos A. P., Discrete Nanostructures of Quantum Dots/Au with DNA. *J. Am. Chem. Soc.* **2004**, (126), 10832-10833.
10. Claridge S.; Liang H W.; Basu S. R.; Fréchet J. M. J.; Alivisatos A. P., Isolation of Discrete Nanoparticle - DNA Conjugates for Plasmonic Applications. *Nanolett.* **2008,** 8, (4), 1202-1206.
11. Park S. Y.; Lytton-Jean A. K. R.; Lee B.; Weigand S.; G., S. C.; Mirkin Ch. A., DNA-programmable nanoparticle crystallization. *Nat.* **2008,** 451, 553-556.
12. Norden B.; Seth S., Structure of Strand-Separated DNA in Different Environments Studied by Linear Dichroism. *Biopol.* **1979,** 18, 2323-2339.
13. Shin M. K.; Kim S. H.; Jung S.; Kim S.I.; Kim S. J.; Kim B. J.; So I., The effect of DNA on mechanical properties of nanofiber hydrogels. *App. Phys. Lett.* **2008,** 93, 171903.

14. Papancea A.; Valente A J. M.; Patachia S.; Miguel M. G.; Lindman B., PVA-DNA Cryogel Membranes: Characterization, Swelling, and Transport Studies. *Langmuir* **2008,** 24, 273-279.
15. Aoi K.; Takasu A.; Okada M., DNA-based polymer hybrids Part 1. Compatibility and physical properties of poly(vinyl alcohol)/DNA sodium salt blend. *Pol.* **2000,** 41, 2847-2853.
16. Rodger A.; Norden B., Circular Dichroism and Linear Dichroism. *Oxf. Univ. Press* **1997**.
17. Cui D.; Pan B.; Zhang H.; Gao F.; Wu R.; Wang J.; He R.; Asahi T., Self-Assembly of Quantum Dots and Carbon Nanotubes for Ultrasensitive DNA and Antigen Detection. *Anal. Chem.* **2008,** 80, (21), 7996–8001.
18. Hammond S.M.; Wood M.J.A., Genetic therapies for RNA mis-splicing diseases. *Tren. in Genet.* **2011,** 27, (5), 196-205
19. Malik R.; Roy I., Making sense of therapeutics using antisense technology. *Exp. Op. on Drug Discov.* **2011,** 6, (5), 507-526
20. Boussif O.; LezoualC'H F.; Zanta M.A.; Mergny M.D.; Scherman D.; Demeneix B.; Behr J.-P., A versatile vector for gene and oligonucleotide transfer into cells in culture and in vivo: Polyethylenimine. *Proc. Natl. Acad. Sci.* **1995,** 92, 7297-7301.
21. Sung S.-J.; Min S.H.; Cho K.Y.; Lee S.; Min Y.-J.; Yeom Y.I.; Park J.-K., Effect of polyethylene glycol on gene delivery of polyethylenimine. *Biol. Pharm. Bullet.* **2003,** 26, (4), 492-500
22. Norden B.; Seth S., Structure of strand-separted DNA in different environments studied by linear dichroism. *Biopol.* **1979,** 18, 2323-2339.
23. Hanczyc P.; Norden B.; Akerman B., DNA in a Poly (Vinyl Alcohol) Matrix and Interactions with Three Intercalating Cyanine Dyes. *J. Phys. Chem. B* **2011,** 115, (42), 12192–12201.
24. Matsuoka Y.; Norden B., Linear Dichroism Studies of Nucleic Acids III/ Reduced Dichroism Curves of DNA in Ethanol-Water and in Poly (vinyl alcohol) Films. *Biopol.* **1983,** 22, 1731-1746.
25. Matsuoka Y.; Norden B., Linear Dichroism Studies of Nucleic Acids. II. Calculation of Reduced Dichroism Curves of A- and B-Form DNA. *Biopol.* **1982,** 21, (12), 2433-2452.
26. Matsuoka Y.; Norden B., Linear Dichroism Studies of Nucleic Acid Bases in Stretched Poly (Vinyl Alcohol) Film. Molecular Orientation and Electronic Transition Moment Directions. *J. Phys. Chem.* **1982,** 86, (8), 1378-1386.
27. Norden B.; Rodger A.; Dafforn T., Linear Dichroism and Circular Dichroism: A Textbook on Polarized-Light Spectroscopy. *Oxf. Univ. Pr.* **2010**.
28. Lindsay S. M.; Lee S. A.; Powell J. W.; Weidlich T.; Demarco C.; Lewen G. D.; Tao N. J.; Rupprecht A., The origin of the A to B Transition in DNA Fibers and Films. *Biopol.* **1988,** 27, 1015-1043.
29. Franklin R. E.; Gosling R. G., The structure of sodium thymonucleate fibres. I. The Influence of water content. *Acta Cryst.* **1953,** 6, 673-677.
30. Falk M.; Hartman K. A.; Lord R. C., Hydration of Deoxyribonucleic Acid. I. a Gravimetric Study. *J. Am. Chem. Soc.* **1962,** 84, 3843-3846.
31. Falk M.; Hartman K. A.; Lord R. C., Hydration of Deoxyribonucleic Acid. II. An Infrared Study. *J. Am. Chem. Soc.* **1962,** 85, 387-391.
32. Tao N. J.; Lindsay S. M.; Rupprecht A., Structure of DNA Hydration Shells Studied by Raman Spectroscopy. *Biopol.* **1989,** 28, 1019-1030.
33. Pilet J.; Brahms J., Investigation of DNA structural changes by infrared spectroscopy. *Biopol.* **1973,** 12, 387-403.

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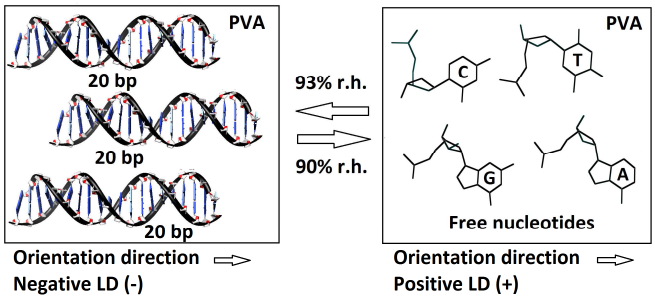
PVA
20 bp
20 bp
20 bp
93% r.h.
90% r.h.
PVA
C
T
G
A
Free nucleotides
Orientation direction
Negative LD (-)
Orientation direction
Positive LD (+)