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Title: Base Inclinations for Synthetic Nucleic Acids in Solution

Abstract Approved:

Dr. W. Curtis Johnson, Jr.

The base inclinations for synthetic nucleic acids in solution are investigated by using flow linear dichroism (LD) extended to the vacuum UV region. Base inclination angles and axes about which the bases incline are derived from the LD, and compared for the deoxyribo-polymers and ribo-polymers in 10 mM phosphate buffer, (pH 7.0), and deoxyribo-polymers under dehydrating conditions. Circular dichroism (CD) spectroscopy is used for monitoring the secondary structural changes for all the polymers in solution. The CD spectra show that the DNA polymers are in the B-form in buffer, while the RNA polymers under same buffer conditions are in the A-form. The inclination angles between the base normal and the helix axis for A-form RNA polymers are larger than for the B-form DNA polymers. Also, the bases in alternating polymers: poly(dAdT)-poly(dAdT), poly(rArU)-poly(rArU), poly(dGdC)-poly(dGdC) and poly(rGrC)-poly(rGrC), usually give larger inclinations than for the corresponding homo-duplex polymers: poly(dA)-poly(dT), poly(rA)-poly(rU), poly(dG)-poly(dC) and poly(rG)-poly(rC).

The CD spectra for the alternating DNA polymers indicate that they undergo a transition from B-form to A-form under dehydrating conditions, and their inclination angles are similar to the corresponding RNA, confirming these results. However, the CD and base inclinations for the homo-duplex DNA polymers in 80% 2,2,2-trifluoroethanol demonstrate that poly(dA)-poly(dT) and poly(dG)-poly(dC) can not form A-DNA under these dehydrating conditions. Their CD spectra have characteristics of both the corresponding B-form DNA and the A-form RNA; their inclination angles and axes are quite similar to the data for the B-form DNA polymers.

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Section I

Introduction

Specific Aims:

Secondary structures of two selected groups of synthetic polynucleotide molecules have been studied and discussed in this work. The double-stranded polymers are: alternating poly(dAdT)-poly(dAdT), poly(rArU)-poly(rArU), poly(dGdC)-poly(dGdC), and poly(rGrC)-poly(rGrC); homoduplex poly(dA)-poly(dT), poly(rA)-poly(rU), poly(dG)-poly(dC), and poly(rG)-poly(rC). The overall aim of this research is to probe the conformation transitions of synthetic polynucleotides and nucleic acids in solution, which are significant and closely related to the expression of their biological functions in the real biology systems. We propose to use vacuum UV linear dichroism (LD) to determine the angles at which the bases are inclined with respect to the helix axis in various base sequences under various solution conditions. Circular dichroism (CD), which is extremely sensitive to the conformation of the biological molecules, also is used for monitoring the secondary structures of these nucleic acids under different solutions conditions. After analysis of the CD, LD, and isotropic absorption (A) spectra, the base inclination angles and axes about which the bases incline for each polymer can be calculated and discussed; and from the overall discussion of

information from both CD and LD studies, we can predict the conformational changes of nucleic acid molecules in response to changes in environmental conditions.

Here we will specifically consider the base inclinations and conformation transitions for groups of synthetic polymers in regular aqueous buffer and under dehydrating conditions. A decrease in the level of hydration in the solution normally forces the DNA structure to undergo a transition from B-form to A-form.

1. Changes in secondary structure:

The vacuum ultraviolet CD spectra of the synthetic polymers under different solution conditions, characterizes the different type of structures that form. We will compare and discuss the CD properties of the different sequences, such as AT polymers and GC polymers; alternating and homoduplex, such as poly(dAdT)-poly(dAdT) and poly(dA)-poly(dT); and B-form DNA polymer and A-form RNA polymer. The goal is to be able to get more information about groups of polymers that will help predict the conformations and stabilities of the polynucleotides in solution.

The CD spectra of all DNA polymers will be measured in both 10 mM sodium phosphate buffer, pH 7.0 and 80% trifluoroethanol (TFE), 0.67 mM sodium phosphate buffer, pH 7.0. Double-stranded DNA polymers in 10 mM sodium buffer should give the B-conformation; RNA polymers normally give the A-form under the same conditions. Dehydrating conditions, such as 80% TFE, is believed to cause the B- to A-form transition in DNA polymers. We

will investigate the behavior of our DNA polymers in 80% TFE, to find out what kinds of transition really happen.

2. Studies of base inclination by LD:

LD spectra will be measured for all the polymers under the same conditions as the CD measurements. Then the LD spectra, along with the isotropic absorption spectra, will be analyzed by using a computerized data analysis method to obtain the base inclination parameters.

3. Effects of solution on nucleic acid structures:

All the results from both LD and CD studies will be discussed and evaluated, and all the structural properties for different polymers under different solution conditions will be compared. The B- to A-form transition will be the focus point. Also, the A-DNA formed will be compared with the native A-RNA. In general, we would like to determine how the solution conditions affect the structures of the synthetic polynucleotides, especially the base inclinations. We hope that these studies contribute to an understanding of the structural features of general nucleic acids in solution. This is essential for understanding their biological functions, since specific sequences of many polymers such as poly(dAdT)-poly(dAdT), poly(dA)-poly(dT) and poly(dGdC)-poly(dGdC) play critical roles in the operation of some biological processes.

Background and Significance:

1. Secondary structures of AT and AU Polymers:

The overall structural properties of alternating poly(dAdT)-poly(dAdT), poly(rArU)-poly(rArU) and homoduplex poly(dA)-poly(dT), poly(rA)-poly(rU) were studied previously in aqueous solution by circular dichroism spectroscopy [ ]. The previous results indicated that under normal physiological conditions the deoxy-AT polymers are in the B-conformation, while the native ribo-AU polymers are in the A-conformation. From comparison of their CD spectra, we could easily distinguish the formation of B- and A-forms. The A-form spectra usually have an intense positive band below 200 nm. The structure transitions of AT polymers were also studied under dehydrating conditions. The results showed that alternating AT polymers can easily go to A-form, but it is difficult for homo poly(dA)-poly(dT) to transform to A-DNA under the same conditions. This was also confirmed by x-ray studies on crystals and fibers of poly(dA)-poly(dT). Recently, many researchers have studied these special polymers and expressed different ideas. In this work, we will investigate this homopolymer by using both CD and LD spectroscopy, and try to find out the secret of this transition.

Previous reports about base inclination studies on AT polymers in low salt buffer indicated that the bases are not perpendicular to the helix axis in solution. There are no previous reports on base orientations for AU polymers in solution. In this work, we will study

the AU polymers structural properties and compare them to the corresponding AT polymers.

Double stranded AT and AU polymers play important roles in many biological system operations and in structure predictions of native nucleic acids in solution. As we know, AT-rich regions are commonly involved in activation and regulation of transcription. Poly(dA)-poly(dT) is involved in dictating translational positioning of DNA on nucleosomes. Oligo(dA)-(dT) sequences are involved in DNA bending. Our study of RNA polymer structure will help to predict the solution conformations and stabilities of real RNA molecules. Therefore, all the structural studies from this work will be very significant.

2. Structures of GC polymers:

Much studies have been devoted to the structure of GC polymers, especially poly(dGdC)-poly(dGdC) since it can be converted into the left handed Z-form. Previous CD results indicated that alternating deoxy- and ribo-GC polymers are sensitive to environmental conditions, such as pH, temperature, solvent, and ionic strength. These polymers can have transitions between the B-, A-, and Z-forms under various solution conditions. Our work will focus on the structural transition of deoxy GC polymers under dehydrating conditions, and compare the base inclinations with the ribo GC polymers through LD measurements.

3. Vacuum UV CD and LD:

CD spectroscopy is particularly sensitive to configuration and conformation of asymmetric biomolecules in solution. Our laboratory has extended CD into the vacuum UV region, which enables us to get more information from the measured spectra. In this work, we use CD spectroscopy to monitor the secondary structure of the polymers and detect directly the conformational transitions in solution.

LD spectroscopy, which also is extended into the vacuum UV region, is a sensitive method for determining the orientation of the bases of polynucleotides in solution. Our LD instrument with a powerful mini pump and flow cell can produce strong LD signals in many different polymers. A more detailed description of LD instrument design and adjustment can be found in the Materials and Methods of section II.

Section II

Comparison of Base Inclination in Ribo-AU and Deoxyribo-AT Polymers

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Abstract

The inclination angle between the base normal and the helix axis is measured for ribo-AU polymers by using flow linear dichroism (LD), and compared to measurements for deoxyribo-AT polymers under dehydrating conditions. The CD of the DNA polymers under the dehydrating conditions is not the same as the corresponding RNA polymers, which are presumed to be in the A-form. However, the LD indicates that poly(dAdT)-poly(dAdT) can assume the A-form in 80% 2,2,2-trifluoroethanol, although poly(dA)-poly(dT) retains its B-form structure in this dehydrating solvent.

Introduction

Much of the information about the structure of nucleic acids comes from X-ray diffraction data on crystals and fibers. However in real biological systems, the biochemical reactions and functional processes in which nucleic acids, polynucleotides, proteins and other biological macromolecules are involved occur in solution. DNA is known to be polymorphic in solution [1-5], and it is not clear how the solution structures relate to the crystal and fiber structures. Therefore, the structure of nucleic acids in a wide range of solutions has also been studied by many researchers. Results of circular dichroism (CD), infrared spectroscopy, fluorescence, and nuclear magnetic resonance indicate that the secondary structure of nucleic acid molecules varies with environmental conditions, such as cation type, temperature, pH, and solvent [1,2,6-15].

We have used flow linear dichroism (LD) extended into the vacuum UV to study base inclinations of natural and synthetic deoxyribo- and ribo-polynucleotides in aqueous solution and in special organic solvents that modify the structure [16-19]. By extending LD measurements into the vacuum UV, we monitor a large number of intense π–π^* transitions with in-plane transition dipoles, so that we have enough information to solve for all of the unknowns, including the orientation factor. Thus we need not extrapolate our LD data to perfect alignment, and any tertiary structure in the sample does not affect our results. A more detailed review of our method and its relationship to the work from other laboratories can be found in Chou and Johnson [20].

In previous work we measured the inclination of poly(dAdT)-poly(dAdT) and poly(dA)-poly(dT) in 10 mM sodium phosphate buffer, pH 7.0, where they are presumably in the B-form [17]. We found that the base normals are fairly inclined with respect to the helix axis: 19 to 23 deg for dA, and 35 to 42 deg for dT. Here we repeat the B-form measurements using new, more sensitive instrumentation, and investigate these polymers under dehydrating conditions where at least poly(dAdT)-poly(dAdT) is believed to assume the A-form. The corresponding ribo polymers are also investigated in buffer where they are presumed to be in the A-form, and the results compared to the deoxyribo polymers. In all cases the inclinations for the ribo polymers are larger than for the corresponding deoxyribo polymers in the B-form. Under the dehydrating conditions of 80% 2,2,2-trifluoroethanol (TFE), poly(dAdT)-poly(dAdT) exhibits A-form inclination, but poly(dA)-poly(dT) retains B-form inclination.

Of course the A-form, B-form, etc. from fiber diffraction of nucleic acids have many defined parameters other than base inclination: number of base pairs per turn; rise per base pair; base tilt , roll, buckle, and propeller twist that combine to give inclination; position of the helix axis relative to the base pair, which in turn determines the grooves; base position relative to the sugar, sugar pucker; etc. Here we measure only the base inclination and the position of the axis around which the base inclines, so the results are only indicative of the form.

Poly(dAdT)-poly(dAdT) is of interest, because the commonly appearing AT-rich regions in DNA molecules are involved in

activation and regulation of gene transcription in real genetic systems [21]. Poly(dA)-poly(dT) is involved in dictating translational positioning of DNA on nucleosomes and affecting the configuration of long runs of free DNA in biological recognition [22-26]. Previous reports indicated that many structural features of this homopolymer are different from other B-type DNA molecules [27]. So far, various studies have indicated that poly(dA)-poly(dT) is unable to assume the A-form. Leslie *et al.* [5] have shown that fibers of poly(dA)-poly(dT) are not affected by environmental changes (such as humidity, ion strength, cation or pH), indicating that it cannot undergo the B- to A-form transition, which can be induced in other DNA sequences under suitable changes in these conditions. An infrared LD study by Pilet *et al.* [11] showed that oriented films of poly(dA)-poly(dT) with 3 to 4% sodium chloride assume two distinct forms, depending on the relative humidity. However, both the high and low humidity forms are interpreted as being in the B-family. Nara-Inui *et al.* [28] used CD to show that the higher the GC content, the easier it is to change natural DNA molecules to the A-form using ethanol as a dehydrating agent. Salt and molecular weight also affected the change. The spectral changes for poly(dAdT)-poly(dAdT) and poly(dA)-poly(dT) were small under these conditions, even at 80% ethanol; the polymers were not believed to have changed into the A-form. However, Vorlíckŏvá *et al.* [29] have used CD to show that they have changed poly(dAdT)-poly(dAdT) into the A-form with 0.02 mM EDTA, 0.15 mM sodium phosphate buffer, pH 6.8, 0 °C, and 71.7% ethanol. Here, our base inclination results indicate that we have changed poly(dAdT)-poly(dAdT) into the A-

form with 80% TFE, but poly(dA)-poly(dT) remains in the B-form, consistent with previous work.

Materials and Methods

Sample preparation

Double-stranded synthetic poly(dA)-poly(dT) (lot CB7860101), poly(dAdT)-poly (dAdT) (lot AE7870111), poly(rA)-poly(rU) (lot AC4521101), and poly(rArU)-poly(rArU) (lot AA7990P03) (MW > 100,000) were purchased from Pharmacia, and were used without further purification. All of the polymers were dissolved in 200 μl of 0.01 M sodium phosphate buffer (from Baker), pH 7.0, at a concentration of about 1 mg (25 OD units) per ml. For spectral measurements in aqueous solution, the concentrated samples were diluted with or dialyzed against 0.01 M sodium phosphate buffer (pH 7.0) to about 0.2 mg (5 or 6 OD units) per ml. Poly(dA)-poly(dT) and poly(dAdT)-poly(dAdT) were dissolved in 80% TFE from Aldrich (> 99% pure) by slowly adding and mixing the TFE with polymer samples in buffer to produce a final sodium phosphate concentration of 0.67 mM. The phosphate buffer maintains the pH at 7.0. The extinction coefficients of poly(dA)-poly(dT) and poly(dAdT)-poly(dAdT) in 0.01 M sodium phosphate buffer were taken to be 6000 L (mol cm)$^{-1}$ at 260 nm, and 6600 L(mol cm)$^{-1}$ at 262 nm, respectively [30]. For poly(rA)-poly(rU) and poly(rArU)-poly(rArU), the extinction coefficients in 0.01 M sodium phosphate buffer were taken to be 6680 L(mol cm)$^{-1}$ and 6100 L(mol cm)$^{-1}$ at 260 nm [31,32]. The extinction coefficients for poly(dA)-poly(dT) and poly(dAdT)-poly(dAdT) in 80% TFE solution were measured to be

6770 L(mol cm)$^{-1}$ at 260 nm, and 5860 L(mol cm)$^{-1}$ at 262 nm, respectively.

Spectral measurements

LD, CD and isotropic absorbance (A) measurements were recorded as described previously [18]. Briefly, the A spectra were recorded at room temperature on a Cary 15 spectrophotometer flushed with nitrogen. Data were collected in every 1 nm from 320 nm to 175 nm. CD spectra were recorded on a modified [33] vacuum-UV MacPherson 225 spectrograph at 20 °C. The CD data were collected every 0.5 nm from 320 nm to 175 nm with an interfaced PC computer. The CD spectrometer was calibrated with (+)-10-camphorsulfonic acid (CSA), assuming $\Delta\varepsilon$ (290.5) = 2.36 and $\Delta\varepsilon$ (192.5) = -4.9. For both CD and A, cylindrical cells (from Hellma) were used, with pathlengths of 0.02 cm, 0.01 cm, or 0.005 cm, depending on the concentration.

Flow LD spectra were measured on the vacuum UV CD spectrometer, but the modulator is now a half-wave retarder, and the frequency of the phase-sensitive detector is doubled. The flow cell system is a stainless-steel Micro Flow-Thro cell (from Barnes) with two quartz windows separated by a teflon spacer to give a pathlength of about 30 μm. A Master-flex L/S micro pump (from Cole-Parmer) with 0.8 mm ID silicon tubing pushed the sample through the cell. This system uses a sample volume as low as 0.6 ml, and the flow rate can be as fast as 36 ml per min (rotor speed 600 rpm). The pumping rate used in these measurements is about 4 ml

per min. Under these conditions a maximum LD signal was achieved, and the polymer samples were oriented with a shear rate of about 14,800 sec^{-1}, calculated as described by Cavalieri *et al.* [34]. The LD calibration was performed with a quartz plate tilted from normal incidence, as described by Norden and Seth [35]. In our LD calibration, a quartz plate tilted at 15° from normal incidence was rotated in four different orientations (0°, 90°, 180°, and 270°) relative to the linear polarization, and LD values in volts were measured from 300 nm to 200 nm, every 20 nm. The wavelength dependence of our measurement was within 2% of the theoretical curve [35], which was used to determine the LD scale factor in LD/volt. The instrument was also stable with time to within ± 2%.

Basic theory

Linear dichroism spectroscopy measures the absorption of linearly polarized light along the principal directions of an anisotropic sample. LD is defined as $A_{||} - A_{\perp}$, where A is the absorbance of the light parallel or perpendicular to the flow (orientation) direction. For a polymer helix that behaves as a homogeneous rod or chain structure, and has only one type of base, the LD as a function of wavelength, λ, is given by [36]:

$$LD(\lambda) = A_{||}(\lambda)-A_{\perp}(\lambda) = \sum_i LD_i(\lambda)$$
$$= \sum_i A_i(\lambda)3S(3\sin^2\alpha\sin^2\beta_i-1)/2 \qquad (1)$$

where α is the inclination angle for the normal to the base plane with respect to the helix axis, and β_i is the angle between the inclination axis and the transition dipole for absorption band i. S is the factor that takes into account the orientation of the sample in the flow ($0 \le S \le 1$), which is the constant for all bands. We do <u>not</u> have to model our flow or extrapolate our data to perfect alignment. The dipole directions are known for all the transitions in our A and LD spectra [37-39], and there are enough bands to determine S experimentally, as well as α and β_i for each type of base. Since the effect of any tertiary structure on preventing perfect alignment can be incorporated into S [36, 40], tertiary structure does <u>not</u> affect our analysis. S is 0.01 to 0.1 for these measurements. However, since S is not important to the analysis, we normalize A and LD to the same

area for convenience of presentation in the figures. For polymers, with more than one base, indexed by j:

$$LD(\lambda) = \sum_{ij} LD_{ij}(\lambda) = \sum_{ij} A_{ij}(\lambda) 3[3\sin^2\alpha_j \sin^2(\chi_j - \delta_{ij}) - 1]/2 \qquad (2)$$

where χ is the angle defining the axis around which the base inclines and δ is the angle defining the transition dipole direction. Both angles are referenced to the C4-C5 bond in the case of purines and the C5-C6 bond in the case of pyrimidines, and measured counter clockwise [41]. It is often convenient to express the data as the reduced dichroism, L, which is the ratio of the LD to the isotropic absorption.

$$L = LD/A = (A_{||} - A_{\perp})/(A_{||} + 2A_{\perp}) \qquad (3)$$

We normalized A and LD to the same area, and plot the normalized reduced dichroism, L', in our figures.

Data analysis

A sophisticated method using the derivative-free Levenberg-Marquardt algorithm (DFLM) has been developed in our laboratory for data analysis of flow LD and absorption spectra to determine base inclinations of nucleic acid molecules in solution [20]. The method uses stability of the parameters, rather than a perfect fit to imperfect data, as the criterion for analysis. This method was used successfully to analyze the LD data of both synthetic polynucleotides

and natural nucleic acids as a function of wavelength for individual base inclinations and axes of inclination. The reliability was tested by reanalyzing LD data of synthetic polymers, which had been analyzed previously by a simpler method; base inclinations from the new sophisticated method are quite similar to the previous ones.

The monomer absorption spectra are fit by using the DFLM algorithm . Generally, several different π -π^* and n-π^* transitions are expected to occur for each base in the measurement region of the LD and absorption spectra between 175 and 300 nm. Our reduced dichroism spectra agreed with previous measurements [17,20], which showed that only the π-π^* transitions contribute to the spectra features. The existence of n-π^* transitions with an out-of-plane dipole direction need not be considered in this work, since they have no significiant contribution. Since we know from other reports the number of $\pi-\pi^*$ bands for each monomer (six for dAMP, three for TMP or UMP), and their approximate position and bandwidth, the monomer absorption spectra can be fitted and decomposed into their constituent bands. A stable decomposition results in four parameters for each band: band center, μ; an integrated intensity, ζ; width at half height, σ; skewness, ρ. These parameters as well as the known in-plane transition dipole directions [37-39], can be found in Table II.1 for A and T. We used these band parameters (with the intensity, ζ, halved to approximate the hypochromism) as the initial guesses for simultaneously fitting the A and LD spectra of each polymer. The transition dipoles for U are assumed to be the same as T [39].

Chou and Johnson [20] give a detailed description of the method for analyzing A and LD spectra of nucleic acids in solution for

Table II.1

Monomer absorption bands and transition dipole directions

base	λ(nm)[a]	ζ x 10^{-3}[b]	σ(nm)[c]	ρ[d]	δ (deg)[e]
adenine	266.4	162.6	11.2	1.20	83
	255.0	319.2	13.9	1.33	25
	206.6	467.0	10.5	1.20	-45
	195.2	78.6	6.1	1.37	15
	184.9	282.2	7.6	1.29	72
	173.6	59.8	4.5	1.00	-45
thymine	265.1	362.4	18.0	1.25	-9
	204.5	400.8	19.3	1.45	-53
	176.6	199.2	6.0	1.46	-26

[a]wavelength maximum of decomposed bands.
[b]intensity of decomposed bands (in units of $nm \cdot L \cdot mol^{-1} cm^{-1}$).
[c]half the bandwidth at half height.
[d]skewness factor.
[e]direction of transition dipole (refs. 36-38).

base inclination, α, and axis of inclination, χ. Since measured A and LD spectra are theoretically related by the inclination angle and axis through equation (2), we fit simultaneously the A and LD spectra with the DFLM algorith and determine the parameters for all bands and inclination angles and axes for the bases in the polymer. Our final chosen fit focuses on minimizing the variance on changing the fitting, and thus achieving stability for all the various parameters. The fitting is very sensitive to the inclination angle, but is insensitive to the position for the inclination axis.

The effect of uncertainties in transition dipole directions for the in-plane $\pi\pi^*$ transitions is also considered during the DFLM analysis. After primary fitting of the spectra with the accepted values of the transition dipole directions, we repeat each fitting 100 times with the transition dipole direction randomly varied within $\pm$ 10 degrees. The final parameters listed in the tables represent the average value of each variable and standard deviations from 100 fittings.

Results and Discussion

We measured the circular dichroism (CD), isotropic absorption (A), and flow linear dichroism (LD) for each of the samples. The CD monitored the conformation of the samples. The A and LD spectra were fit simultaneously, as described in the Materials and Methods section, to obtain the inclination of the base normal from the helix axis (α), the orientation of the axis around which the base is inclined (χ), and the fraction of sample oriented (S). Many workers express their linear dichroism data as the reduced dichroism (L), and we plot a normalized reduced dichroism (L') in the figures for convenience.

Poly(dAdT)-poly(dAdT) and poly(rArU)-poly(rArU)

Figure II.1 shows the CD spectrum of poly(dAdT)-poly(dAdT) in a 10 mM sodium phosphate buffer, pH 7.0, where it is presumed to be in its normal B form. This is the typical spectrum with a positive band at about 260 nm, a negative band at about 250 nm, and an intense positive band below 200 nm, which is consistent with earlier work [6,17,42]. The measured A and LD spectra, normalized to the same area, and the calculated L' spectrum are given in Figure II.2a. These are similar to earlier measurements from our laboratory [17]. The results of decomposing the absorption into the six-component bands for dA and the three-component bands for dT are given in Table II.2, along with the α and χ angles that result from simultaneously fitting the LD spectrum. Decomposition of both A and LD for poly(dAdT)-poly(dAdT) are shown in Figure II.3, but the

Figure II.1: The circular dichroism of poly(dAdT)-poly(dAdT) in 10 mM sodium phosphate buffer, pH 7.0 (----), poly(dAdT)-poly(dAdT) in 80% 2,2,2-trifluoroethanol, 0.67 mM sodium phosphate buffer, pH 7.0 (------), and poly(rArU)-poly(rArU) in 10 mM buffer, pH 7.0 (— — —).

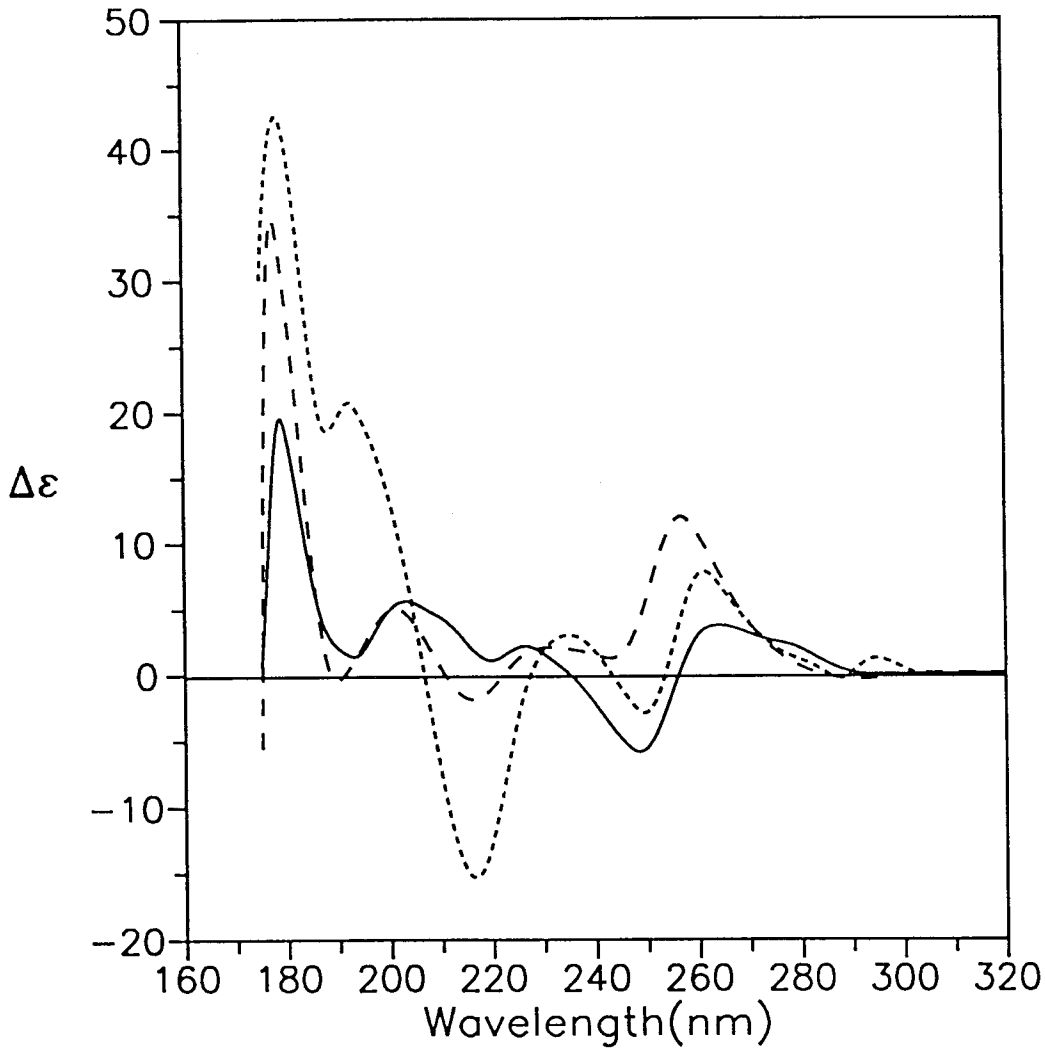

50
40
30
20
10
0
−10
−20
Δε
160
180
200
220
240
260
280
300
320
Wavelength(nm)

Figure II.1

Figure II.2a. The normalized isotropic absorption (A) in absorbance units (–––––), normalized flow linear (LD) dichroism with sign reversed (— — —), and normalized reduced linear dichroism (L') (------) for poly(dAdT)-poly(dAdT) in 10 mM sodium phosphate buffer, pH 7.0.

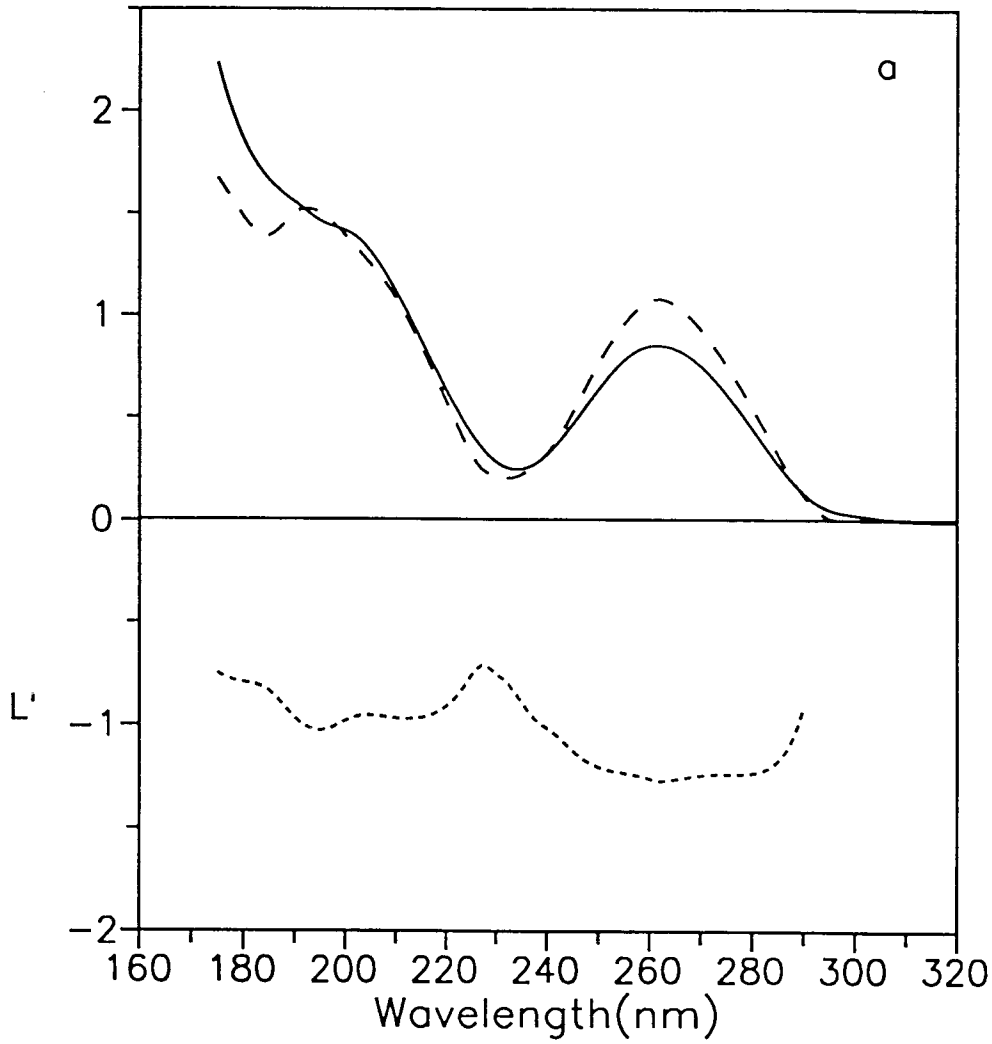


Figure II.2a

Figure II.2b. The normalized A in absorbance units (----), normalized LD with sign reversed (— — —), and normalized L' (------) spectra for poly(rArU)-poly(rArU) in 10 mM sodium phosphate buffer, pH 7.0.

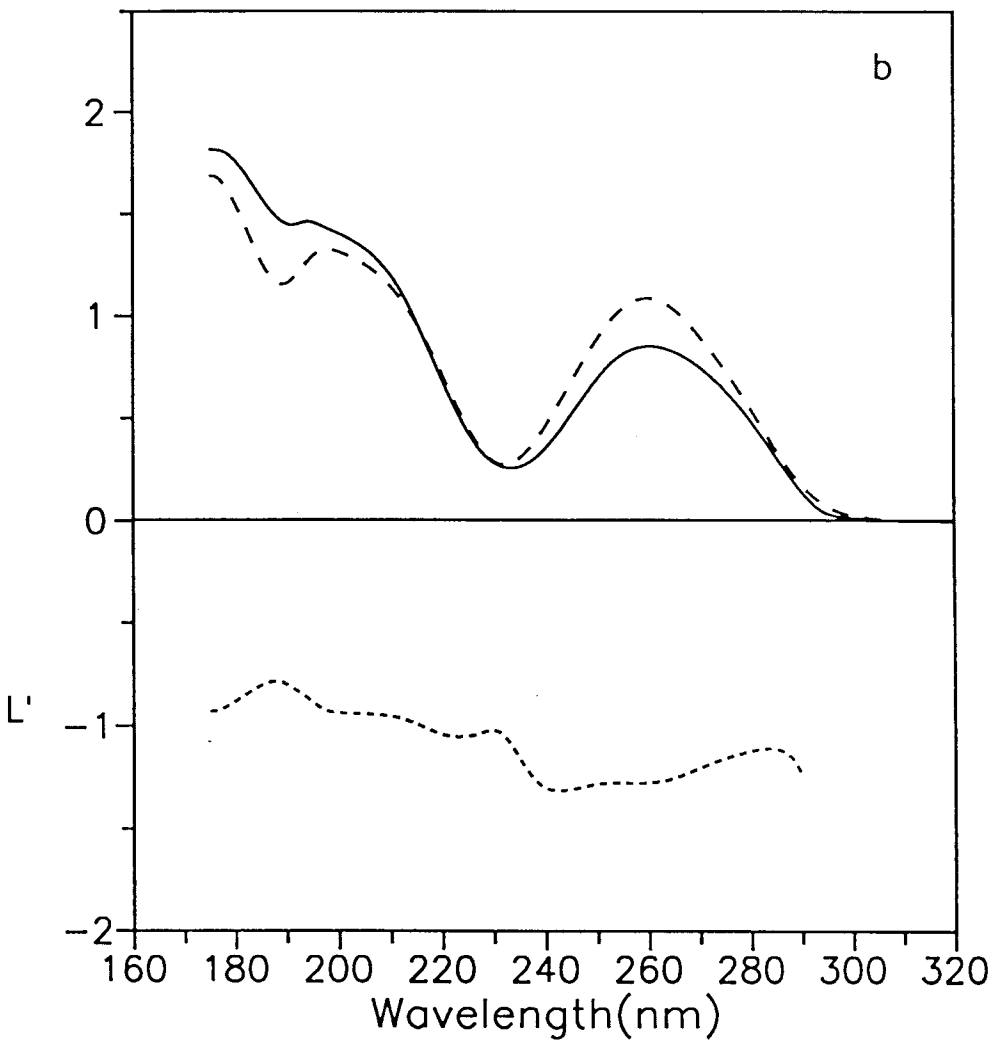


Figure II.2b

Figure II.2c. The normalized A in absorbance units (----), normalized LD with sign reversed (— — —), and normalized L' (------) spectra for poly(dAdT)-poly(dAdT) in 80% TFE, 0.67 mM sodium phosphate buffer, pH 7.0.

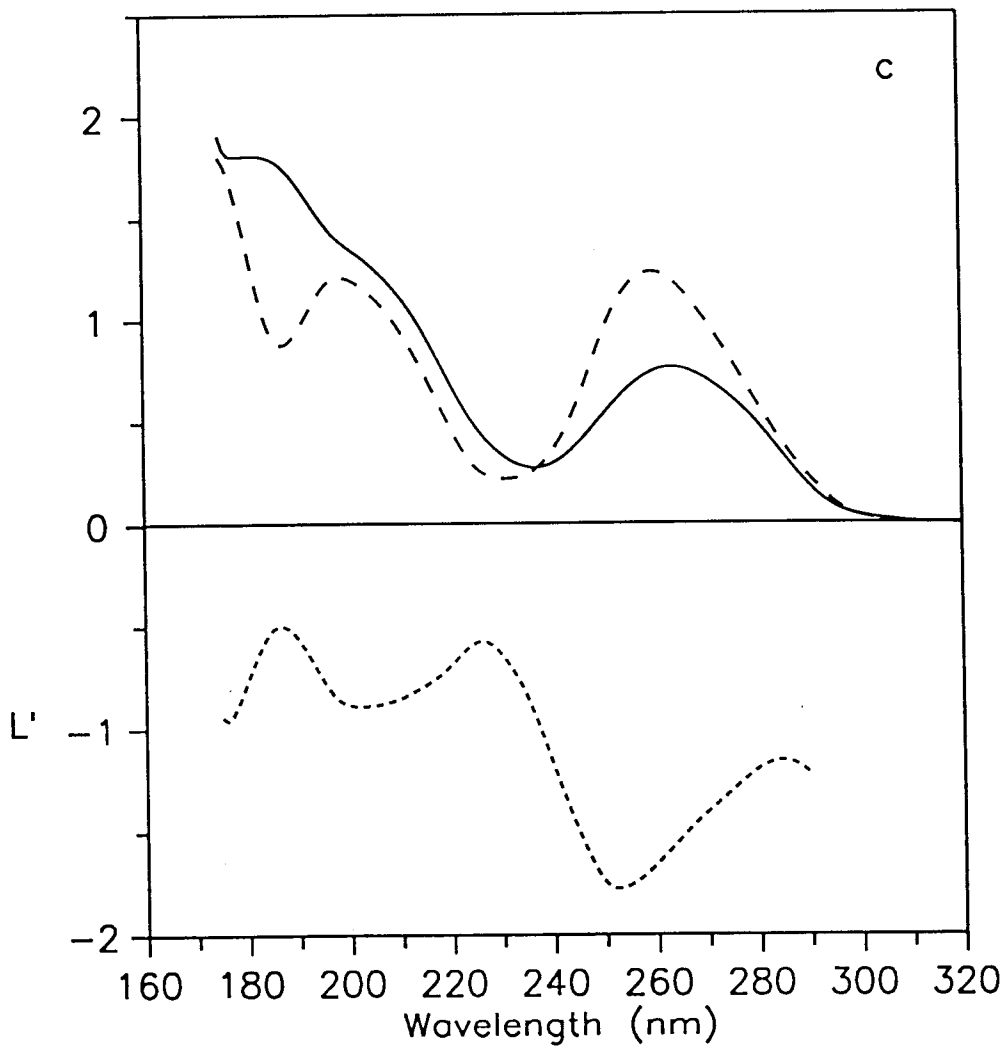


Figure II.2c

Table II.2

Absorption bands, inclination angles (α), and axes of inclination (χ) for alternating copolymers[a]

Base	μ(nm)	ζ x 10^{-3}	σ(nm)	ρ	α(deg)	χ(deg)
Poly(dAdT)-poly(dAdT) in buffer						
dA	272.7 ± 0.4	44.1 ± 2.2	15.5 ± 0.4	1.50 ± 0.00	17.6 ± 1.0	-7.2 ± 4.5
	257.2 ± 0.5	85.5 ± 1.2	13.0 ± 0.3	1.01 ± 0.01	(18.6 ± 0.6)	(-16.1 ± 3.4)
	206.4 ± 0.1	172.5 ± 2.5	13.1 ± 0.1	1.00 ± 0.01		
	195.6 ± 0.1	43.4 ± 1.3	6.4 ± 0.0	1.01 ± 0.01		
	184.9 ± 0.1	116..4 ± 1.6	7.8 ± 0.1	1.23 ± 0.03		
	173.0 ± 0.1	24.7 ± 2.3	3.0 ± 0.1	1.03 ± 0.05		
dT	268.7 ± 1.2	109.4 ± 1.0	17.1 ± 0.9	1.26 ± 0.03	37.1 ± 2.0	18.1 ± 3.4
	196.2 ± 2.1	175.3 ± 6.8	27.6 ± 0.7	1.05 ± 0.02	(34.8 ± 2.0)	(18.7 ± 3.2)
	176.1 ± 0.2	97.4 ± 2.4	5.3 ± 0.1	1.01 ± 0.01		
Poly(rArU)-poly(rArU) in buffer						
rA	274.6 ± 0.3	42.9 ± 1.7	12.0 ± 0.3	1.27 ± 0.08	23.0 ± 1.1	-9.2 ± 3.5
	254.2 ± 0.1	90.9 ± 0.4	13.1 ± 0.2	1.17 ± 0.03		
	208.4 ± 0.3	159.7 ± 3.1	13.2 ± 0.1	1.01 ± 0.01		
	196.5 ± 0.1	28.3 ± 2.6	6.4 ± 0.1	1.02 ± 0.01		
	184.9 ± 0.2	106.2 ± 0.7	8.3 ± 0.1	1.00 ± 0.02		
	173.5 ± 0.1	26.7 ± 1.4	3.4 ± 0.1	1.02 ± 0.01		

(Table II.2, continued)

rU	267.4 ± 0.4	113.3 ± 1.7	17.8 ± 0.1	1.17 ± 0.01	35.7 ± 2.3	6.4 ± 5.6
	199.9 ± 0.8	163.9 ± 3.0	21.5 ± 0.8	1.37 ± 0.03		
	177.5 ± 0.1	75.4 ± 0.7	6.0 ± 0.0	1.07 ± 0.01		
Poly(dAdT)-poly(dAdT) in 80% TFE						
dA	276.1 ± 0.5	45.3 ± 6.5	10.3 ± 0.4	1.06 ± 0.02	32.2 ± 1.1	-4.5 ± 2.6
	255.8 ± 0.2	90.9 ± 3.2	12.1 ± 0.3	1.21 ± 0.06		
	206.5 ± 0.5	177.5 ± 10.2	13.4 ± 0.3	1.01 ± 0.02		
	196.6 ± 0.3	30.3 ± 4.3	7.1 ± 0.3	1.01 ± 0.02		
	186.3 ± 0.2	146.2 ± 3.8	7.3 ± 0.3	1.00 ± 0.04		
	173.7 ± 0.2	36.4 ± 3.3	3.1 ± 0.2	1.01 ± 0.01		
dT	268.1 ± 0.7	121.6 ± 4.8	16.3 ± 0.3	1.27 ± 0.08	41.9 ± 1.6	6.9 ± 3.6
	202.9 ± 2.4	224.3 ± 10.2	26.7 ± 2.5	1.18 ± 0.08		
	177.6 ± 0.2	94.5 ± 3.8	5.6 ± 0.2	1.11 ± 0.03		

[a]Symbols as in Table II.1.

Figure II.3: Decomposition of the normal isotropic absorption (top) and flow linear dichroism (bottom) spectra for poly(dAdT)-poly(dAdT) in 10 mM sodium phosphate buffer, pH 7.0. The bands due to dA (— — —) and the bands due to dT (------) combine to give the fitted spectrum (————) that is compared to the measured spectrum (□ □ □).

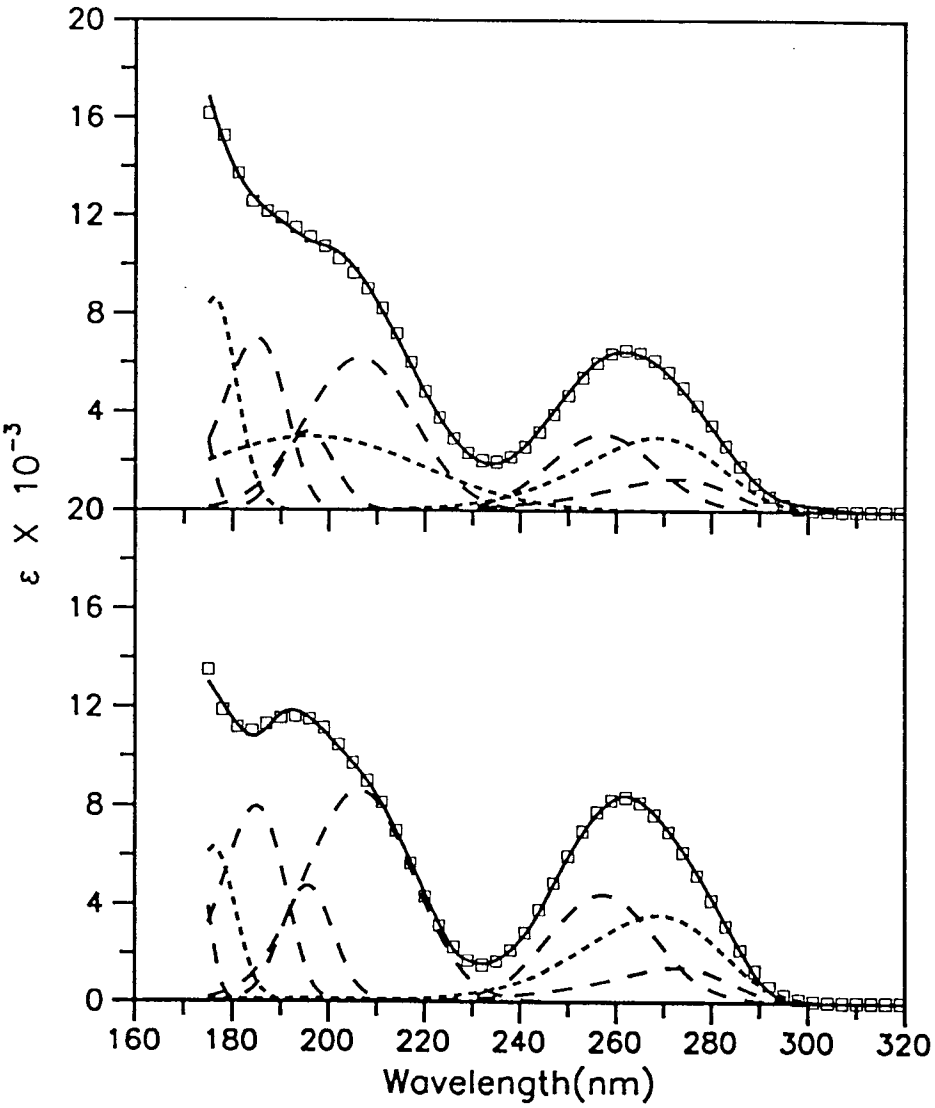

20
16
12
8
4
20
16
12
8
4
0
ε X 10^-3
160
180
200
220
240
260
280
300
320
Wavelength(nm)

Figure II.3

results obtained for subsequent data will not be shown. These α and χ angles are similar to the values obtained from earlier work [17] using the new algorithm and the updated transition dipole directions [20], which are given in Table II.2 in the parentheses. They differ somewhat from the α and χ angles in Edmondson and Johnson [17], primarily because the absorption of dA is now decomposed into six component bands, rather than four. The α angles are accurately determined, and are fairly insensitive to LD data. However, the χ angles are quite sensitive to the LD data, so the position of the inclination axis must be considered an estimate. The measured inclination angle of 17.6° for dA in B-form poly(dAdT)-poly(dAdT) is similar to the value that we have measured for natural B-form DNA, but the inclination angle of 37.1° for dT is about 12° larger than the value measured for natural B-form DNA [20].

Poly(rArU)-poly(rArU) in 10 mM sodium phosphate buffer, pH 7.0, is presumably in the A form, which is the standard form for an RNA. The CD spectrum shown in Figure II.1 is typical for the A form, with a positive band around 260 nm and an intense positive band below 200 nm, in agreement with previous work [31]. LD data presented in Figure II.2b are new. The shape of the L' spectrum for poly(rArU)-poly(rArU) is quite different from that of poly(dAdT)-poly(dAdT) in buffer, displaying a remarkable variation with wavelength. The parameters for decomposition of the absorption spectrum, and the α and χ angles for simultaneous decomposition of the LD spectrum are presented in Table II.2. The measured inclination angle of 23.0° for rA in A-form poly(rArU)-poly(rArU) is somewhat larger than the corresponding value of 17.6° for dA in B-

form poly(dAdT)-poly(d(AdT), but the inclination angle of 35.7° for rU is virtually the same as the value for dT. Although the measured LD for the DNA and RNA polymers in buffers are quite different, the inclination angles for the bases are not really very different. Furthermore, the position of the axes around which the bases incline are similar for the two polymers in buffer.

Dehydrating alcohol solvents, such as TFE, can cause double-stranded DNA polymers to assume the A form [3,4,6,11,43]. While it is more difficult to transform DNA polymers with a high AT content into the A form [28], it can certainly be done for the alternating poly(dAdT)-poly(dAdT) [11,29]. The CD of poly(dAdT)-poly(dAdT) in 80% TFE, 0.67 mM phosphate buffer, pH 7.0, is compared to the B form in buffer and A form poly(rArU)-poly(rArU) in Figure II.1. This CD spectrum does not resemble the CD of poly(rArU)-poly(rArU) as might be expected if it were in the A form. However, the 260 nm band for poly(dAdT)-poly(dAdT) in 80% TFE is more intense than is measured for the B form, and it does have an intense negative band at 218 nm and a large positive intensity below 200 nm, as is typical of the A-form of natural DNAs.

The LD results, which are new, are given in Figure II.2c. Here we see that the L' spectrum differs significantly from both B-form poly(dAdT)-poly(dAdT) and A-form poly(rArU)-poly(rArU). The parameters for decomposition of the absorption, and the α and χ angles for simultaneous fitting of the LD are given in Table II.2. Although the L' spectrum is not particularly varied, the analysis gives large inclination angles of 32.2° for dA and 41.9° for dT. These A-form inclination angles for poly(dAdT)-poly(dAdT) are

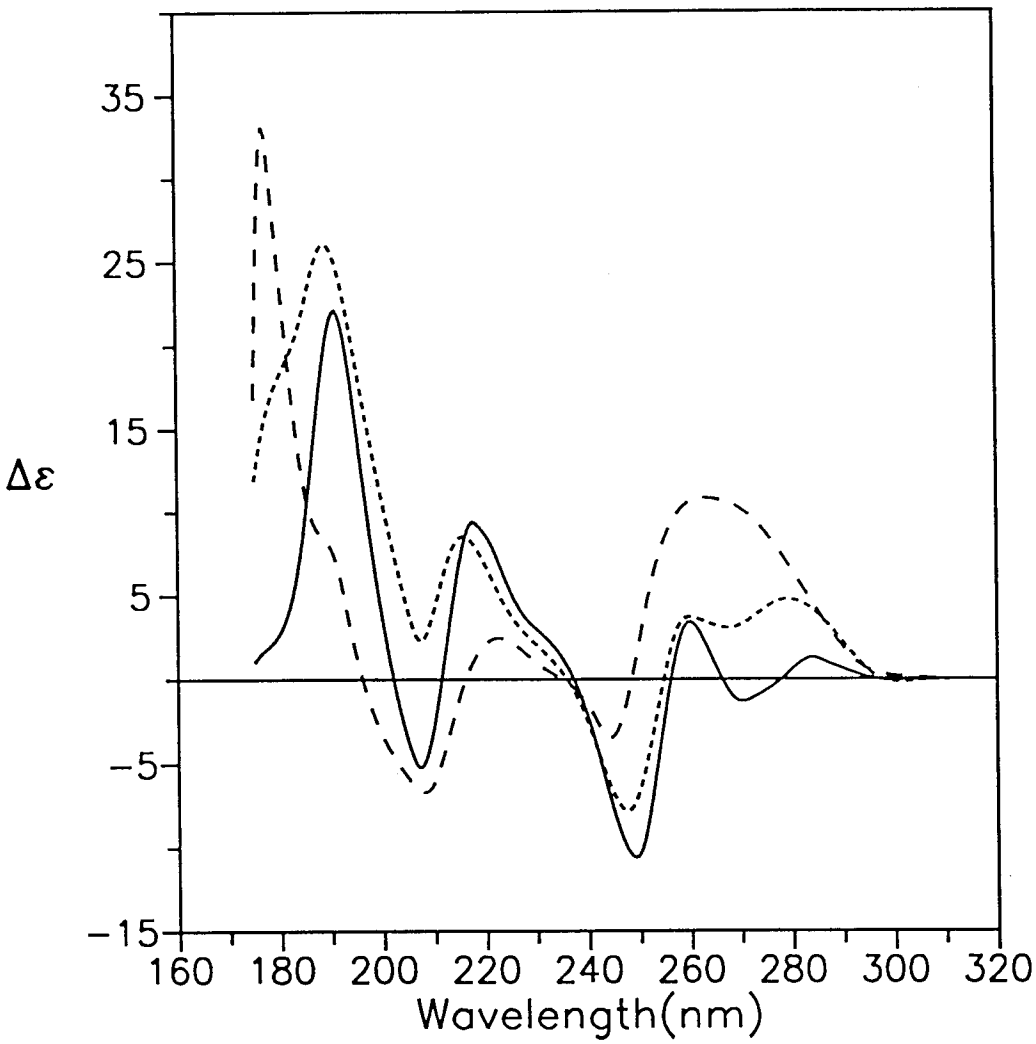
considerably larger than the B-form angles, and are even significantly larger than the A-form angles for poly(rArU)-poly(rArU). However, the axes of inclination, which are not accurately determined from our data, are similar in all three cases. The base dA in the A-form of natural DNA has an α angle intermediate between A-form dA and rA found here, while dT in A-form natural DNA has an α angle similar to rU [20].

Both the CD and L' spectra indicate that poly(dAdT)-poly(dAdT) in 80% TFE has a conformation that is different from A-form poly(rArU)-poly(rArU) and our LD results show that corresponding inclination angles are indeed different. While the CD and L' spectra give the impression that poly(dAdT)-poly(dAdT) has not attained the A form in 80% TFE, our LD results give the inclination angles that are larger for the DNA polymer under dehydrating conditions than they are for the RNA polymer, indicating that poly(dAdT)-poly(dAdT) has indeed attained the A form in 80% TFE.

Poly(dA)-poly(dT) and poly(rA)-poly(rU)

The CD spectrum of double-stranded poly(dA)-poly(dT) in 10 mM sodium phosphate buffer, pH 7.0, where it is presumed to be in its normal B form, is shown in Figure II.4. In agreement with previous results [7,42,44], the spectrum is rich in bands and has a very intense positive band at about 190 nm. The A, LD, and L' spectra of B-form of poly(dA)-poly(dT) measured here are given in Figure II.5a. They are nearly identical to the spectra measured earlier in this laboratory [17]. They were analyzed to give the band

Figure II.4: The circular dichroism of poly(dA)-poly(dT) in 10 mM sodium phosphate buffer, pH 7.0 (----), poly(dA)-poly(dT) in 80% 2,2,2-trifluoroethanol, 0.67 mM sodium phosphate buffer, pH 7.0 (------), and poly(rA)-poly(rU) in 10 mM sodium phosphate buffer (— — —).


35
25
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−15
Δε
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180
200
220
240
260
280
300
320
Wavelength(nm)

Figure II.4

Figure II.5a. The normalized A in absorbance units (-----), normalized LD with sign reversed (— — —), and normalized L' (------) spectra for poly(dA)-poly(dT) in 10 mM sodium phosphate buffer, pH 7.0.

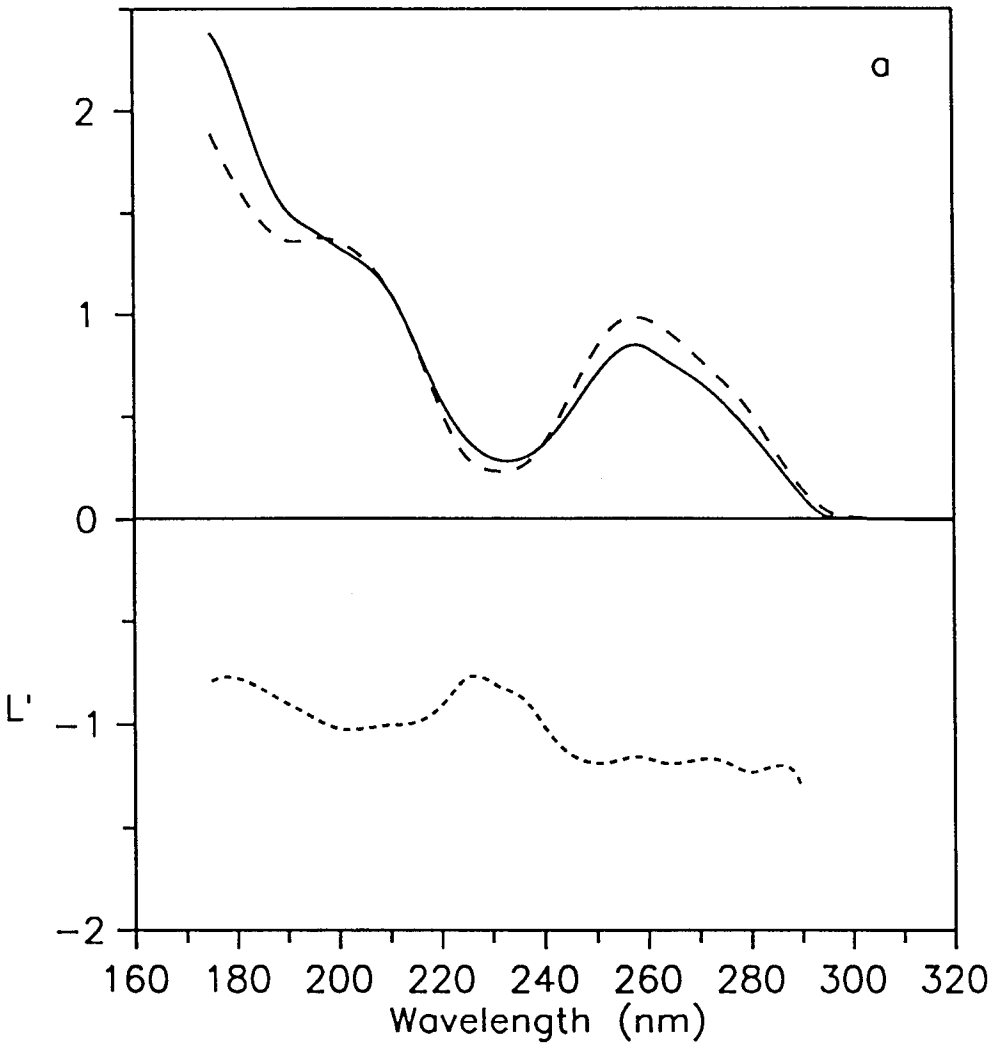

Figure II.5a

Figure II.5b. The normalized A in a absorbance units (-----), normalized LD with sign reversed (— — —), and normalized L' (------) spectra for poly(rA)-poly(rU) in 10 mM sodium phosphate buffer, pH 7.0.

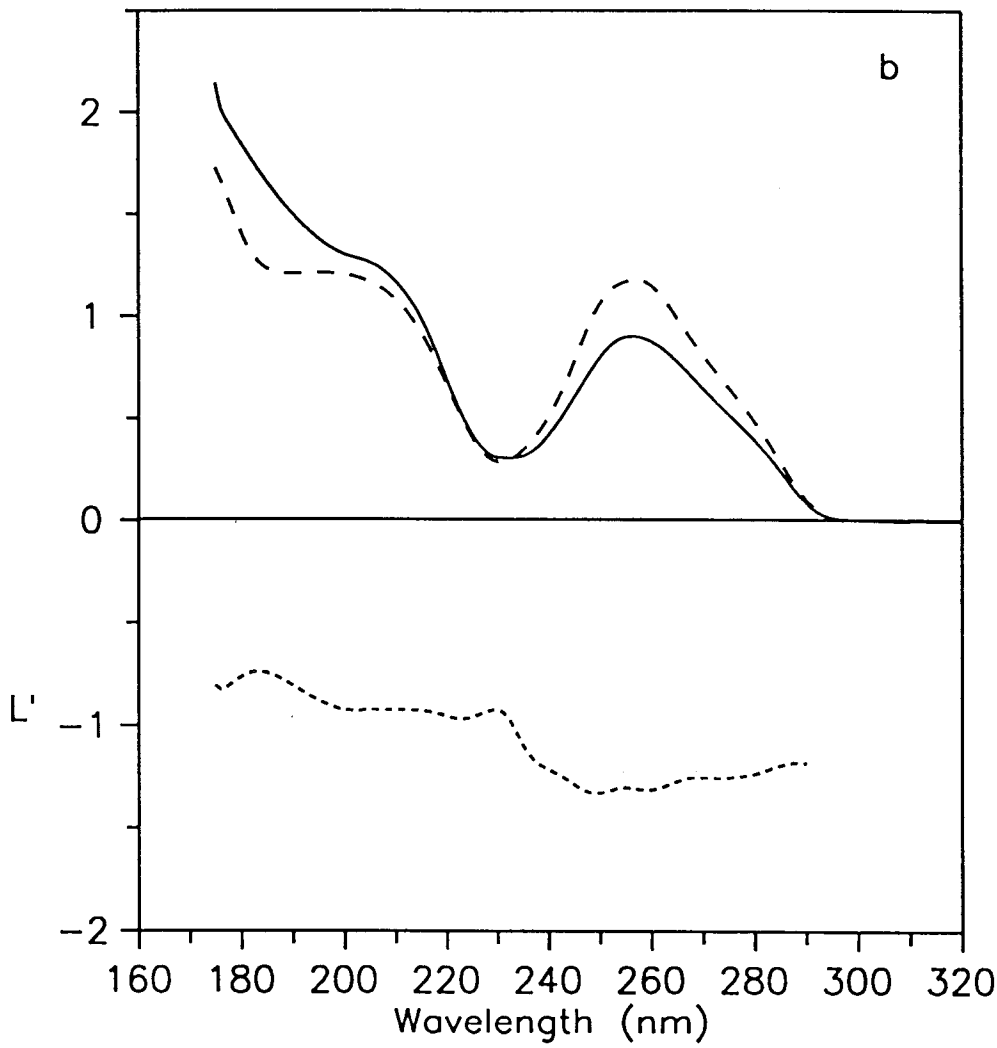

Figure II.5b

Figure II.5c. The normalized A in a absorbance units (–––––), normalized LD with sigh reversed (— — —), and normalized L' (------) spectra for poly(dA)-poly(dT) in 80% TFE, 0.67 mM sodium phosphate buffer, pH 7.0.

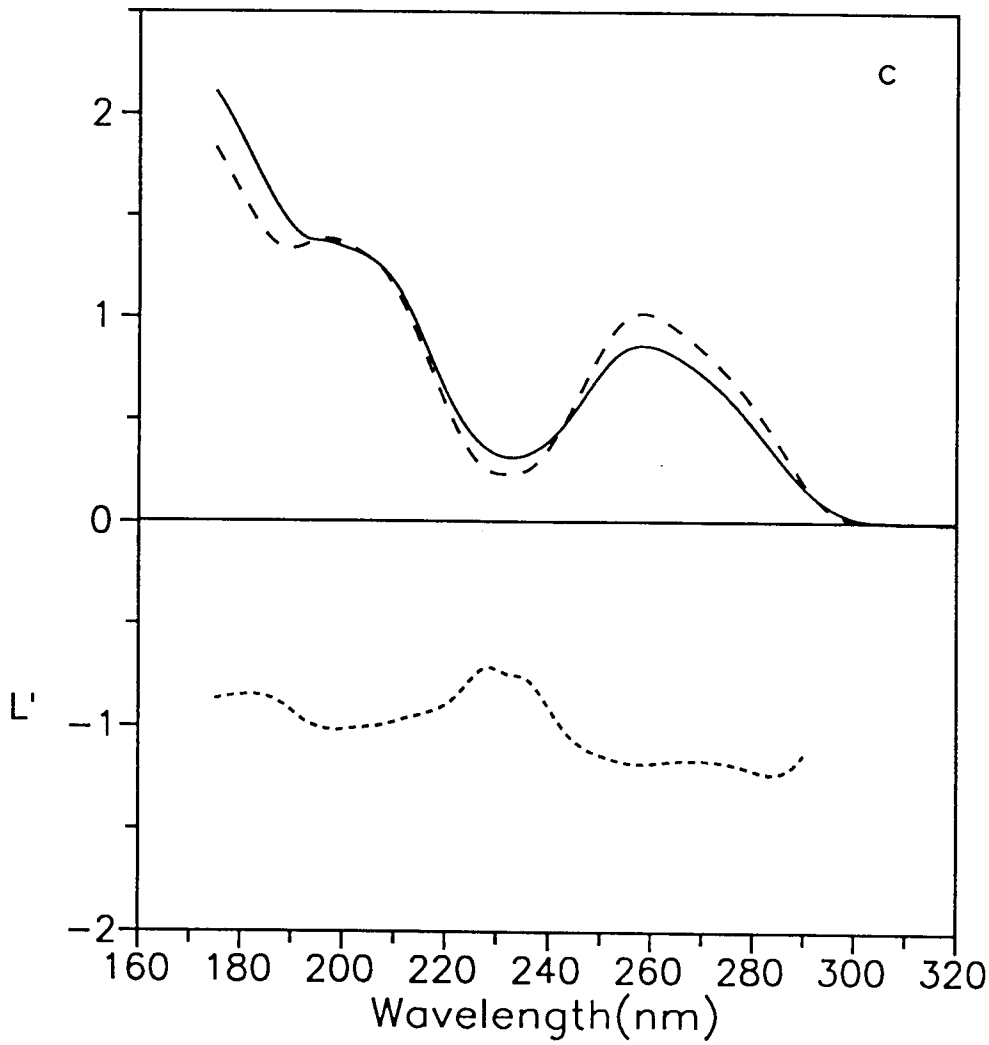

Figure II.5c

parameters, and α and χ angles in Table II.3. Inclinations of 18.5° for dA and 37.3° for dT are about 5° lower than we obtained using the new algorithm on earlier data [20], as shown in the parentheses in Table II.3. The inclination angle for dA is considerably lower than the inclination angle obtained by Edmondson and Johnson [17], because we are now using six bands rather than four bands in the decomposition of the adenine absorption. These B-form inclination angles for the double-stranded homopolymer are nearly identical to the inclination angles for the double-stranded alternating polymers, shown in Table II.2. Again, although the dA inclination is similar to that for natural B-form DNA, the α for dT is about 12° larger [20].

The CD of poly(rA)-poly(rU) in 10 mM sodium phosphate buffer, pH 7.0, is also shown in Figure II.4. This is a typical A form spectrum with an intense positive band at about 260 nm, a negative band at about 210 nm, an intense positive CD below 200 nm. Corresponding linear dichroism data (A, LD, and L') are new, and given in Figure II.5b. The data do not look much different from B-form data for poly(dA)-poly(dT) in Figure II.5a; this is confirmed by the analysis shown in Table II.3. The inclination for rA at 23.7° is about five degrees larger than dA in the B-form polymer, but the inclination angle of 37.7° for rU is about the same as dT. The inclination axes have nearly the same orientation for the DNA and RNA polymers in buffer.

The CD spectrum of poly(dA)-poly(dT) in 80% TFE solution does not resemble the CD of poly(rA)-poly(rU), as one might expect for the A-form. This result agrees with the previous measurement by Nara-Inui *et al.* [28]. The shape of the CD speactrum still has

Table II.3

Absorption bands, inclination angles (α), and axes of inclination (χ) for homopolymers[a]

Base	μ(nm)	$\zeta \times 10^{-3}$	σ(nm)	ρ	α(deg)	χ(deg)
Poly(dA)-poly(dT) in buffer						
dA	273.2 ± 0.8	39.6 ± 6.3	14.5 ± 0.8	1.12 ± 0.03	18.5 ± 0.6	-24.3 ± 4.1
	254.1 ± 0.3	84.2 ± 1.4	11.6 ± 0.2	1.00 ± 0.01	(23.2 ± 0.8)	(-28.4 ± 3.7)
	205.8 ± 0.2	157.2 ± 3.6	12.7 ± 0.2	1.01 ± 0.01		
	195.1 ± 0.2	35.3 ± 1.2	6.3 ± 0.1	1.04 ± 0.01		
	183.9 ± 0.2	119.6 ± 2.4	7.4 ± 0.1	1.14 ± 0.02		
	172.6 ± 0.1	21.3 ± 2.3	2.8 ± 0.1	1.06 ± 0.06		
dT	269.6 ± 2.9	108.6 ± 3.5	18.6 ± 1.2	1.50 ± 0.00	37.3 ± 3.0	14.5 ± 4.3
	194.8 ± 1.6	164.5 ± 8.3	27.6 ± 0.0	1.01 ± 0.05	(42.1 ± 2.5)	(21.1 ± 3.2)
	175.6 ± 0.3	103.3 ± 5.0	5.2 ± 0.2	1.03 ± 0.01		
Poly(rA)-poly(rU) in buffer						
rA	270.8 ± 0.8	42.5 ± 1.5	14.3 ± 0.4	1.40 ± 0.13	23.7 ± 0.6	-8.3 ± 2.2
	253.9 ± 0.1	102.6 ± 0.9	12.6 ± 0.1	1.41 ± 0.06		
	207.5 ± 0.1	166.5 ± 2.6	12.6 ± 0.1	1.06 ± 0.01		
	195.3 ± 0.1	32.1 ± 1.4	6.4 ± 0.1	1.21 ± 0.05		
	184.9 ± 0.1	115.0 ± 0.7	7.5 ± 0.1	1.17 ± 0.01		
	173.9 ± 0.2	29.7 ± 1.7	3.1 ± 0.1	1.02 ± 0.05		
rU	267.4 ± 0.4	122.6 ± 1.4	17.8 ± 0.2	1.30 ± 0.02	37.7 ± 1.7	14.7 ± 3.0
	201.1 ± 1.1	168.9 ± 6.0	23.5 ± 0.8	1.45 ± 0.03		
	176.9 ± 0.1	81.8 ± 0.5	5.5 ± 0.0	1.10 ± 0.01		

(Table II.3, continued)

Poly(dA)-poly(dT) in 80% TFE						
dA	272.4 ± 2.3	36.3 ± 4.1	16.4 ± 2.9	1.29 ± 0.22	18.9 ± 0.8	-13.0 ± 3.2
	254.1 ± 3.6	79.1 ± 3.6	11.5 ± 5.2	1.12 ± 0.03		
	206.1 ± 0.1	164.4 ± 5.1	12.5 ± 5.2	1.01 ± 0.01		
	194.9 ± 0.4	35.5 ± 2.3	6.0 ± 0.2	1.02 ± 0.01		
	184.1 ± 0.4	109.9 ± 1.8	7.4 ± 0.0	1.22 ± 0.02		
	172.8 ± 0.2	21.8 ± 2.9	3.0 ± 0.1	1.06 ± 0.10		
dT	270.5 ± 0.5	112.2 ± 4.8	16.0 ± 0.6	1.21 ± 0.4	35.6 ± 3.4	10.9 ± 4.8
	199.2 ± 6.0	167.6 ± 18.0	31.5 ± 3.0	1.01 ± 0.01		
	176.0 ± 0.4	89.7 ± 1.1	5.5 ± 0.1	1.01 ± 0.00		

[a]Symbols as in Table II.1.

some similarity to the B-form in buffer, but the intensity and position of the CD bands are changed somewhat.

The A, LD and L' spectra of poly(dA)-poly(dT) in 80% TFE, 0.67 mM sodium phosphate buffer (pH 7.0) are new and are shown in Figure II.5c. The shape of the normalized, reduced LD spectrum looks very similar to the one for the B-form polymer in sodium phosphate buffer, and indeed the analysis is also similar. Table II.3 shows that the inclinations of 18.9° for dA and 35.6° for dT are virtually the same as for the B-form in buffer. The data are less sensitive to the axes of inclination, which are not significantly different for the two solvents. Clearly, poly(dA)-poly(dT) is still in the B-form in 80% TFE.

Acknowledgment

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Section III

Comparison of Base Inclination in Synthetic Ribo-GC and Deoxyribo-GC Polymers

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Key words: Linear dichroism, base tilt, A-form, GC polymers

Abstract

The inclination angle between the base normal and the helix axis is measured for ribo-GC polymers by using flow linear dichroism (LD), and compared to measurements for deoxyribo-GC polymers under dehydrating conditions. The CD of poly(dGdC)-poly(dGdC) under the dehydrating conditions is similar to poly(rGrC)-poly(rGrC), indicating it is in the A-form, and analysis of the LD confirms this interpretation. A new method was designed to synthesize poly(rGrC)-poly(rGrC), which is not available commercially, in large quantities. The CD of poly(dG)-poly(dC) under dehydrating conditions has characteristics of both the B-form DNA and the corresponding A-form RNA. Analysis of the LD indicates that poly(dG)-poly(dC) under dehydrating conditions has the bases in the B-form orientation.

Introduction

The structure of nucleic acids has been a research target for decades, even since Watson and Crick discovered the double-stranded helical structure of DNA [1]. In recent years, more information has been obtained about the structure of DNA molecules in crystals and fibers through X-ray diffraction [2-5]. The solution structures of nucleic acids, which may not be directly predictable from their crystal or fiber structures, depend on the variation of environmental conditions, such as solvent, pH, temperature, and ion strength [6-17]. However, the structure of nucleic acids in solution is more difficult to obtain, although a number of spectroscopic techniques have been used to investigate aspects of nucleic acid structure in various solutions [11-15].

One spectroscopic technique, linear dichroism (LD) spectroscopy is particularly well suited to measure base inclinations of nucleic acids in solution [18-20]. Flow linear dichroism has been used in our laboratory to study natural DNA, and synthetic ribo- and deoxyribo-polynucleotides under various solvent conditions [21-23]. In order to collect more structural information through LD measurements, we extend the flow LD scan into the vacuum UV to 175 nm. Then the LD data is analyzed by using our new algorithm as described in Chou and Johnson [24]. With the larger information content in our extended flow LD data, we can determine the inclination of the base normal from the helix axis, the axis around which the base inclines, and the orientation factor for the polymer in the flow for each base.

We do not have to extrapolate our data to perfect alignment, and any tertiary bending will not affect our analysis.

Circular Dichroism (CD) also is used in this work as a supporting technique to monitor the secondary structure of the nucleic acids, and any conformation transitions as the solution conditions change [25]. The structural change investigated here is the B-form to A-form transition as the solvent is changed from a low salt aqueous buffer to the dehydrating organic solvent [8,26].

Several synthetic deoxyribo-GC and ribo-GC polynucleotide molecules are investigated by measuring their CD, LD and isotropic absorption (A) spectra in both 10 mM phosphate buffer, pH 7.0, and in 80% 2,2,2-trifluoroethanol (TFE). The alternating poly(dGdC)-poly(dGdC) and homo-duplex poly(dG)-poly(dC) are presumably in the B-conformation in 10 mM sodium phosphate buffer, pH 7.0. The alternating poly(rGrC)-poly(rGrC) and homo-duplex poly(rG)-poly(rC) as ribo-polymers are in the native A-form in the same buffer conditions. CD measurements confirm this interpretation, and agree with the previous reports [27-30].

Base inclinations for B-form poly(dGdC)-poly(dGdC) and poly(dG)-poly(dC) have been studied in the early work from our laboratory [27]. The results showed that the base normals in both deoxy-GC polymers are fairly inclined with respect to their helix axis, about 20 degrees for G and 24 to 29 degrees for C. In this work we remeasure the LD spectrum for those B-form deoxy-GC polymers under same the solution conditions with increased instrumental sensitivity. Also, we analyze the measured spectra with the new algorithm [24]. The base inclinations for the A-form ribo-

polynucleotides, poly(rGrC)-poly(rGrC) and poly(rG)-poly(rC), as measured in buffer, show a larger inclination. TFE, a dehydrating alcohol, is believed to induce double-stranded DNA molecules into the A-form [26,31]. Here, we measure the CD, LD, and A spectra of deoxy-GC polymers in 80% TFE, 0.67 mM phosphate buffer, pH 7.0, and compare the their CD spectra and base inclinations with the results for A-form RNA polymers.

Alternating poly(dGdC)-poly(dGdC) is an interesting polymer with a secondary structure that is very sensitive to salt concentration, pH, and solvent [29,30]. Earlier work indicates that poly(dGdC)-poly(dGdC) is in the regular B-form in 10 mM phosphate buffer, in the A-form in 80% TFE and even in the Z-form in high salt buffers, such as 2M $NaClO_4$ [30].

The corresponding alternating poly(rGrC)-poly(rGrC) is not commercially available, as was synthesized in large scale in our laboratory using a new *in vitro* transcription catalyzed by T7 RNA polymerase. The secondary structure of this RNA polymer in solution, which is normally the A-form, changes to the Z-form in 6 M $NaClO_4$ at 46 ^{o}C [30]. Here the CD spectrum and base inclinations of A-form poly(rGrC)-poly(rGrC) have been taken as the standard to compare with the data for the A-form poly(dGdC)-poly(dGdC) in 80% TFE.

The homo-duplexes poly(dG)-poly(dC) and poly(rG)-poly(rC) are also compared. These polymers have slightly smaller inclination angles than the alternating polymers. Also, the differences among B-form poly(dG)-poly(dC), A-form poly(rG)-poly(rC) and A-form poly(dG)-poly(dC) in 80% TFE are not significantly different.

However, CD spectra and base inclination angles for poly(dG)-poly(dC) in 80% TFE are quite similar to the A-form poly(rG)-poly(rC) [28]. Therefore, poly(dG)-poly(dC) indeed becomes A-DNA in 80% TFE.

Materials and Methods

Sample preparation

Synthetic homo-duplex poly(dG)-poly(dC) (lot AA7890P11) and alternating poly(dGdC)-poly(dGdC) (lot BH7910104, approximate average length of 805 base pairs) were purchased from Pharmacia. Synthetic RNA homo-duplex poly(rG)-poly(rC) was purchased from Sigma. All three polymers were used without further purification. Both poly(dG)-poly(dC) and poly(rG)-poly(rC) were dissolved and dialyzed against 10 mM sodium phosphate buffer (from Baker), pH 7.0, to give a final concentration of about 1 mg (25 OD units) per ml. Ten OD units of poly(dGdC)-poly(dGdC) powder were dissolved in 0.40 ml of deionized water (0.625 mg per ml). Then, the poly(dGdC)-poly(dGdC) sample was dialyzed against 1 L of 0.5M NaCl, 10 mM sodium-EDTA, pH 8.0, for about 8 hr; dialyzed against 1 L of 0.01M NaCl, 0.01M sodium-EDTA, 2 mM sodium phosphate buffer, pH 8.0, for another 8 hr; and finally dialyzed twice against 10 mM sodium phosphate buffer, pH 7.0, for a total of 12 hr. The multi-step dialysis performed on poly(dGdC)-poly(dGdC) helps remove possible contaminating multivalent impurities in the polymer sample, since multivalent ions (like Ca^{2+}, Mg^{2+}) may produce the Z or Z' form of poly(dGdC)-poly(dGdC) under the dehydrating conditions used here to produce the A-form.

The extinction coefficients for poly(dG)-poly(dC) at 260 nm and poly(dGdC)-poly(dGdC) at 256 nm in a low concentration of phosphate buffer were taken to be 7060 $L(mol\ cm)^{-1}$ and 7100

L(mol cm)$^{-1}$, respectively [27]. The extinction coefficients of poly(dGdC)-poly(dGdC) and poly(dG)-poly(dC) in 80% TFE were measured here to be 6650 L(mol cm)-1 at 258 nm and 7500 L(mol cm)$^{-1}$ at 260 nm. For the A-form of poly(rG)-poly(rC), the extinction coefficient in 10 mM sodium phosphate buffer was taken to be 7460 L(mol cm)$^{-1}$ at 260 nm [28].

Alternating poly(rGrC)-poly(rGrC) is not commercially available, and was synthesized using either bacteriophage T7 RNA polymerase or *Escherichia coli* RNA polymerase, and a poly(dIdC)-poly(dIdC) template. We tested both T7 and *E.coli* RNA polymerase for *in vitro* synthesis, and found that T7 RNA polymerase is much more efficient for the rapid large-scale synthesis in vitro of poly(rGrC)-poly(rGrC). T7 RNA polymerase, which is a single-subunit enzyme produced by bacteriophage, was purchased from US Biochemical with a high concentration of 80 units per microliter. *E. coli* RNA polymerase was also purchased from US Biochemical (Specific activity of 900 to 1000 units/mg). Poly(dIdC)-poly(dIdC) (average length about 1,100 bp), rCTP, and rGTP were purchased from Pharmacia, and were used without further purification. DNase I (Bovine pancreas) was purchased from Worthington (ribonuclease-free, specific activity about 3,139 units/mg dry protein). Distilled water, buffers and reagents used in the synthesis and purification were autoclaved or specially treated to prevent ribonuclease contamination. The procedures for large-scale *in vitro* synthesis using T7 RNA polymerase were developed as part of this research. Synthesis using *E.coli* RNA polymerase was a modification of Hall *et al.*(1985). A detailed description and discussion of the poly(rGrC)-

poly(rGrC) synthesis are presented in the Results and Discussion section of this paper. The extinction coefficient at 260 nm for poly(rGrC)-poly(rGrC) in low salt phosphate buffer was taken to be 6560 L(mol cm)$^{-1}$ [30,32].

For spectral measurements, all the stock solutions were diluted with 10 mM sodium phosphate buffer, pH 7.0, to 5 or 6 OD per ml (about 200 μg per ml). The A-form of alternating poly(dGdC)-poly(dGdC) and homo-duplex poly(dG)-poly(dC) were obtained by slowly mixing 2,2,2-trifluoroethanol (TFE, >99% pure, from Aldrich) with the polymer solution to 80% (v/v) of TFE concentration. The ribonucleotide polymers poly(rG)-poly(rC) and poly(rGrC)-poly(rGrC) gave the characteristic native A-form CD in 10 mM sodium phosphate buffer, pH 7.0.

Spectral measurements and Analysis

Details of the instrumentation, the basic theory of linear dichroism, and our method for analysis of the data are given in the accompanying paper describing our research on ribo-AU and deoxy AT-polymers.

Results and Discussion

Alternating poly(rGrC)-poly(rGrC) was synthesized in large-scale *in vitro* by transcription catalyzed by RNA polymerase, which provided sufficient RNA polymer for our structural studies. In this work, we measure the circular dichroism (CD), isotropic absorption (A), and flow linear dichroism (LD) for the synthetic GC polymers: poly(dG)-poly(dC), poly(dGdC)-poly(dGdC), poly(rG)-poly(rC) and poly(rGrC)-poly(rGrC). We analyze both A and LD spectra simultaneously to determine the inclination of the base normal from the helix axis (α), the axis around which the base inclines (χ), and the fraction of sample oriented in our flow cell (S). We also plot the normalized reduced dichroism (L') for convenience. We compare base inclination results for deoxy-GC polymers in the normal B-form and ribo-GC polymers in the normal A-form to the deoxy-GC polymers under dehydrating conditions, where they presumably assume the A-form.

Large-scale *in vitro* synthesis of poly(rGrC)-poly(rGrC)

Producing poly(rGrC)-poly(rGrC), which is not available commercially, through large-scale *in vitro* synthesis was essential for comparison with A-form poly(dGdC)-poly(dGdC). Chamberlin *et al.* [33] have shown that under optimal reaction conditions RNA polymerase can remain active for a long period of time, and an RNA polymer can be synthesized in quantities suitable for physical studies. Hall *et al.* [34] established the optimal conditions for

extensive synthesis of poly(rGrC)-poly(rGrC) using *E. coli* RNA polymerase with poly(dIdC)-poly(dIdC) as the template. We developed synthesis procedures for using both *E.coli* RNA polymerase and bacteriophage T7 RNA polymerase. T7 RNA polymerase is believed to need a promoter to be effective, while *E. coli* RNA polymerase does not need a promoter [35,36]. However, T7 RNA polymerase proved much more effective than *E.coli* RNA polymerase, even though our poly(dIdC)-poly(dIdC) template has no specific promoter.

The synthesis of poly(rGrC)-poly(rGrC) by using *E. coli* RNA polymerase follows a procedure modified from Hall *et al.* [34]. Since the specific activity and unit definition of the RNA polymerase used here are different from that used by Hall *et al.*, the optimal reaction conditions for the poly(rGrC)-poly(rGrC) *in vitro* synthesis were investigated using various concentrations of RNA polymerase, which were estimated to have approximately equal activity to the enzyme originally used. The optimal synthesis conditions using RNA polymerase from the U S Biochemical were found to be 40 mM Tris buffer, pH 8.0; 0.5 mM $MnCl_2$; 0.5 mM spermidine; 10 mM ß-mercaptoethanol; 2 μM poly(dIdC)-poly(dIdC); 2 mM rGTP; 2 mM rCTP; and 10 units/ml RNA polymerase. The reactions were carried out at 37 ^{o}C for 24 hr in reaction volumes of 1 ml. Fresh template was added at 2 μM to each 1 ml volume every 4 hr to ensure its effective concentration. For larger scale synthesis, we usually used five to ten 1-ml volumes. However, the final yield of RNA polymer by this method was not in the large-scale necessary for our LD measurements.

The optimized reaction conditions for the *in vitro* synthesis using T7 RNA polymerase was 40 mM Tris buffer, pH 8.0; 15 mM $MgCl_2$; 5 mM dithiothritol; 0.5 mg/ml BSA (nuclease free); 2 mM rGTP; 2 mM rCTP; 0.1 mM poly(dIdC)-poly(dIdC); and 2.8 units/μl T7 RNA polymerase in total reaction volumes of 1 ml. The synthesis reaction was in progress at 37 °C for 1 to 1.5 hrs, compared to 24 hrs for the *E.coli* RNA polymerase. Then, the 1 ml mixture was heated to 70 °C for about 10 min to melt synthesized RNA from the template. After cooling slowly to room temperature, DNase I (RNase free, working concentration 3 units/μl) digestion was carried out at 25 °C for 1 hr in the presence of 5 mM $CaCl_2$. Two phenol extractions and three chloroform:isoamyl alcohol (24:1) extractions removed the protein. To remove the digested DNA and change solvent, the sample was dialyzed three times against 20 ml autoclaved (RNase free) 10 mM sodium phosphate buffer, pH 7.0. After dialysis, the yield of poly(rGrC)-poly(rGrC) was calculated from its normal UV absorption.

The size distribution and purity of the poly(rGrC)-poly(rGrC) was detected by a 5% polyacrylamide gel with 8 M urea to separate the strands, and the synthesized product labeled by γ-^{32}P-ATP. Native gel electrophoresis with either 5% polyacrylamide or 2% agarose was also used for monitoring the lengths of the RNA polymers obtained after synthesis. The synthetic poly(rGrC)-poly(rGrC) molecules usually ranged from 100 basepairs to 500 basepairs or even longer. In order to estimate the efficiency of the synthesis with T7 RNA polymerase, we tested the incorporation of α-^{32}P-rGTP into the oligonucleotides. A small amount of α-^{32}P-rGTP was added to a small volume of reaction mixture that included

buffer, rGTP, rCTP, template and T7 RNA polymerase. After incubating at 37 °C for 0.5 hr, 1 hr, and 2 hr, the samples collected at different time points were run through Thin-Layer-Chromatography (TLC). The autoradiogram showed that almost 100% of the labeled GTP molecules were incorporated to the synthetic oligomers under the reaction conditions after 1 hr. Therefore, the *in vitro* large-scale of poly(rGrC)-poly(rGrC) by T7 RNA polymerase is much more efficient and faster than the method catalyzed by E. coli polymerase.

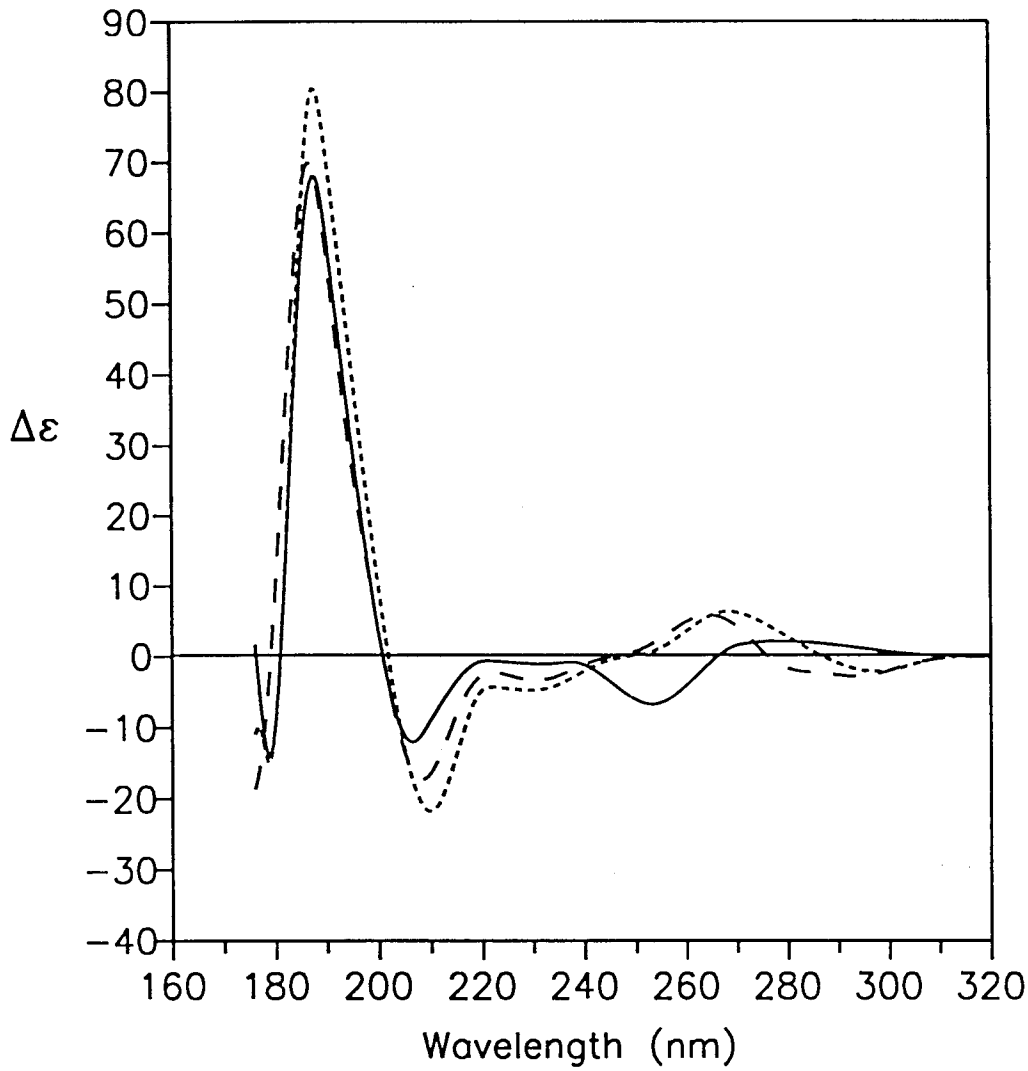
We could easily get enough RNA polymer for the spectroscopic studies carried out in this work. Compared with *E.coli* RNA polymerase synthesis, the final yield of poly(rGrC)-poly(rGrC) from T7 RNA polymerase reaction was 25 OD units per ml, or 1.25 mg per 1 ml reaction mixture (about 6 fold higher than *E.coli* RNA polymerase method). The synthetic poly(rGrC)-poly(rGrC) was characterized by absorption spectroscopy and CD spectroscopy before it was used for further LD studies.

Poly(dGdC)-poly(dGdC) and poly(rGrC)-poly(rGrC)

The CD spectrum of poly(dGdC)-poly(dGdC) in 10 mM sodium phosphate buffer, pH 7.0, which gives the B-conformation, is shown in Figure III.1. This spectrum contains a small flat positive band around 280 nm, two negative bands at 253 nm and 207 nm, and an typical intense positive band at 188 nm, in agreement with previous work [27].

In order to calculate the base inclination of this alternating GC polymer in buffer, we also measure the A and LD spectra in the same

Figure III.1: The circular dichroism of poly(dGdC)-poly(dGdC) in 10 mM sodium phosphate buffer, pH 7.0 (–––––), poly(dGdC)-poly(dGdC) in 80% 2,2,2-trifluoroethanol, 0.67 mM sodium phosphate buffer, pH 7.0 (------), and poly(rGrC)-poly(rGrC) in 10 mM buffer, pH 7.0 (— — —).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Wavelength (nm)

Figure III.1

wavelength range. The normalized A and LD, as well as the normalized reduced LD (L'), are shown in Figure III.2a, and are very similar to the earlier measurements [27]. The A and LD spectra are simultaneously decomposed into four bands for dG and five bands for dC, which represent the different components at different wavelengths (Figure III.3). Other decompositions will not be shown in figures. Table III.2 lists the decomposition data for each of the component bands, as well as the inclination angle (α) and its axis (χ) that relate the LD to A. The inclination angle of 25.5^o at an axis of 136.6^o for dG and 32.5^o at an axis of 217.6^o for dC in the B-form poly(dGdC)-poly(dGdC) are close to the results from previous measurements, although here we are using a new algorithm [24], and more accurate transition dipole directions (see Table III.1) [37,38]. The old values for α and χ [24,27] are listed in Table III.2 in parentheses. Comparison between the two sets of data shows that the α angles are more stable than the χ values, as expected, because α angles are better determined by the LD data than the χ angles [41]. Therefore, base inclination angles obtained from spectra decomposition and fitting are quite accurate, but angles for axes of inclination are an estimate.

The alternating RNA poly(rGrC)-poly(rGrC), which was synthesized in large-scale by our own efforts, is studied in 10 mM phosphate buffer by measuring its CD, A and LD spectra. Since the RNA polymer normally gives the A-form conformation in low salt aqueous buffer, we can use it as a standard to compare with the structure of poly(dGdC)-poly(dGdC) in the A-form under dehydrating conditions. The CD spectrum of poly(rGrC)-poly(rGrC) in 10 mM

Figure III.2a. The normalized isotropic absorption (A) in absorbance units (----), normalized flow linear (LD) dichroism with sign reversed (— — —), and normalized reduced linear dichroism L' (------) for poly(dGdC)-poly(dGdC) in 10 mM sodium phosphate buffer, pH 7.0.

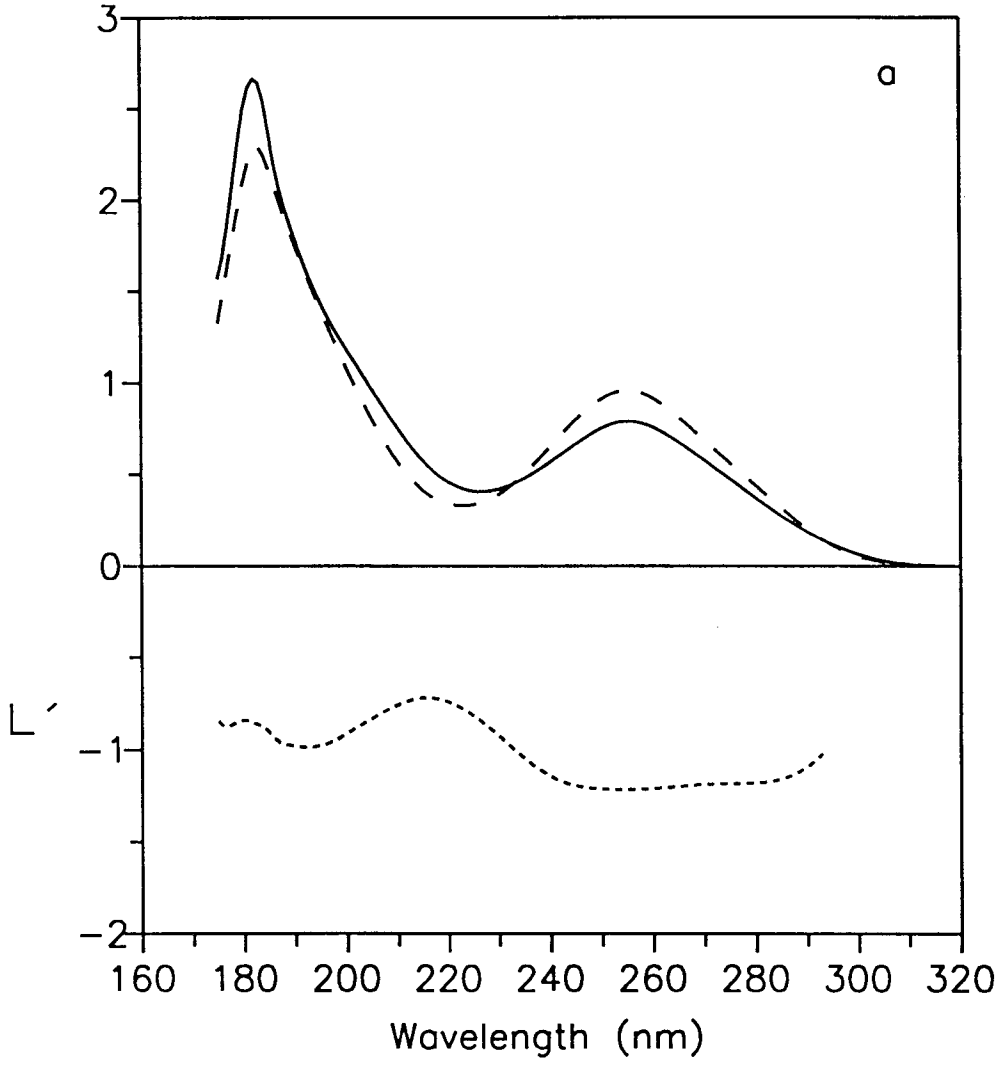


Figure III.2a

Figure III.2b. The normalized A in absorbance units (–––––), normalized LD with sign reversed (— — —), and normalized reduced linear dichroism L' (------) spectra for poly(rGrC)-poly(rGrC) in 10 mM sodium phosphate buffer, pH 7.0.

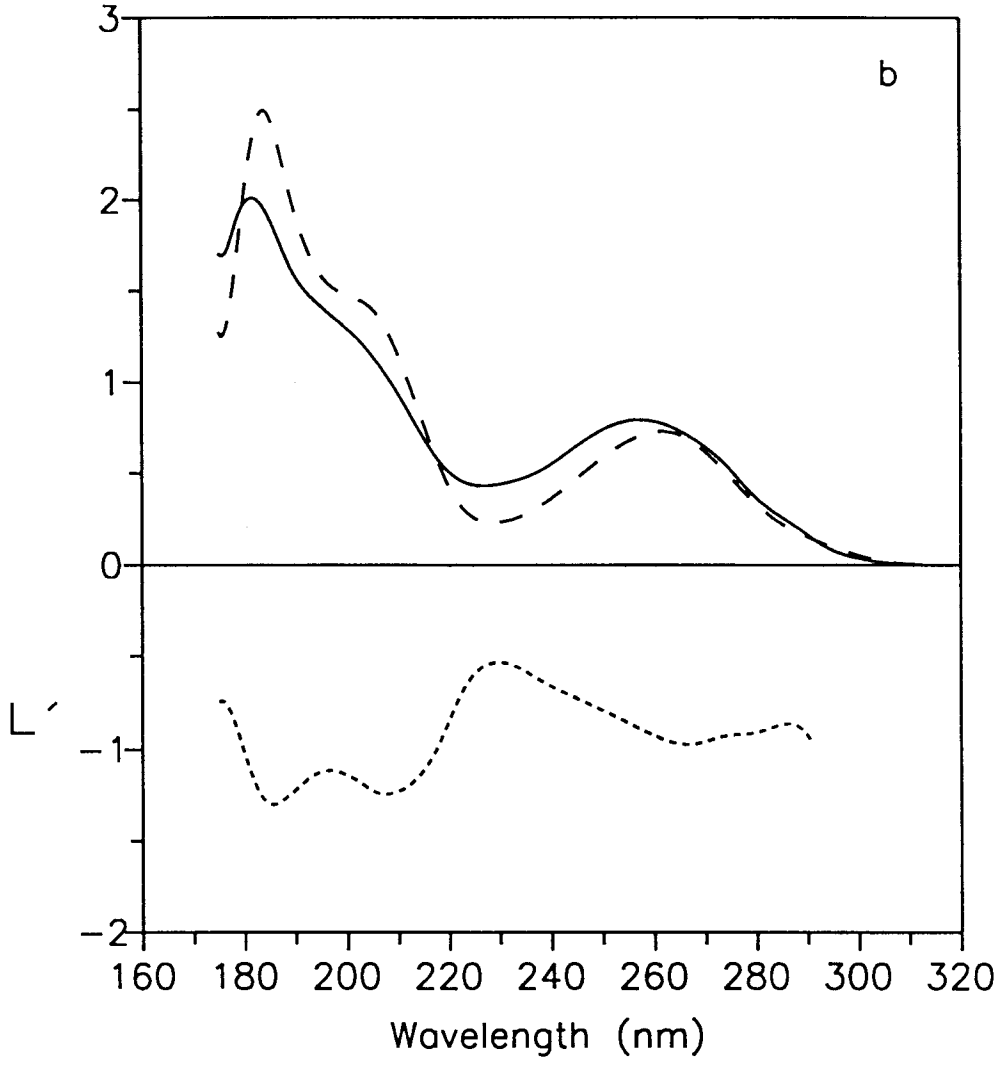


Figure III.2b

Figure III.2c. The normalized A in absorbance units (–––––), normalized LD with sign reversed (— — —), and normalized reduced linear dichroism L' (------) spectra for poly(dGdC)-poly(dGdC) in 80% TFE, 0.67 mM sodium phosphate buffer, pH 7.0.

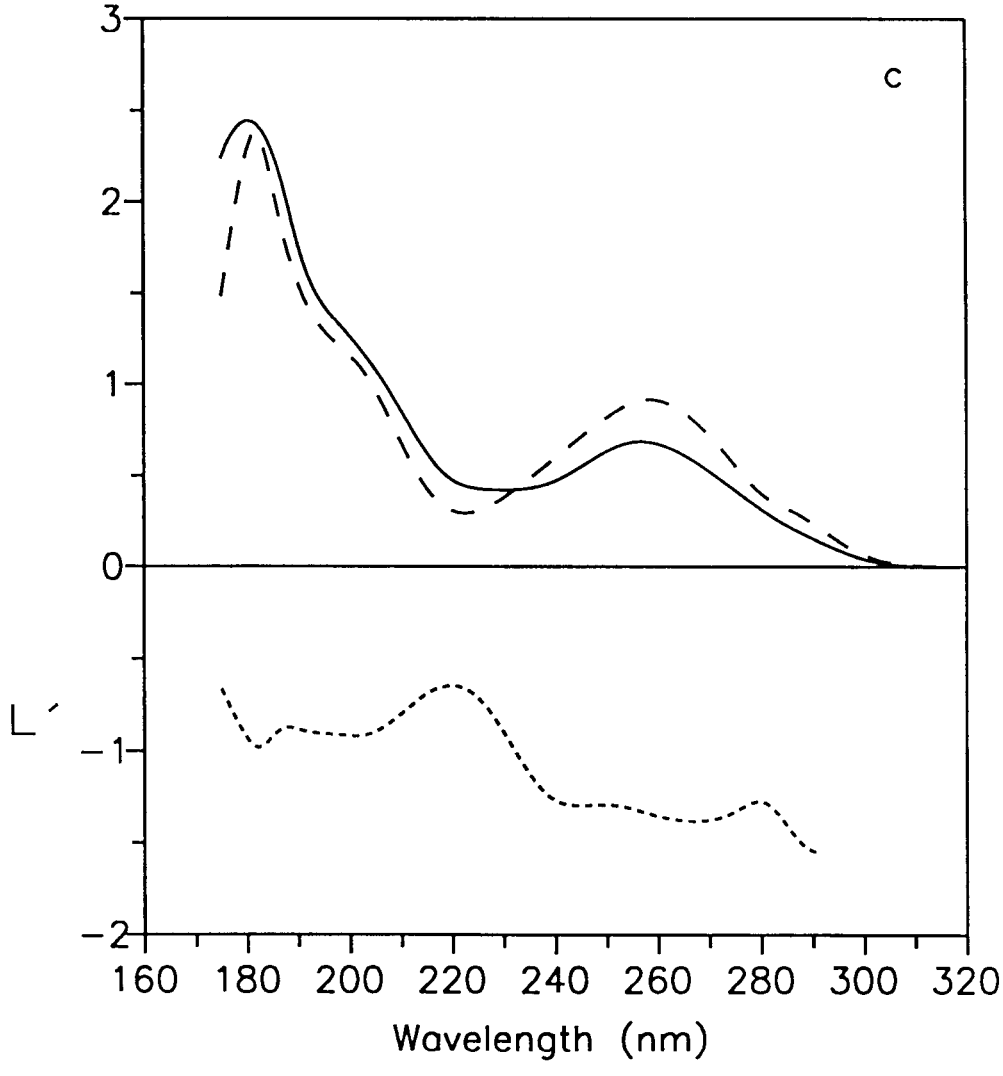
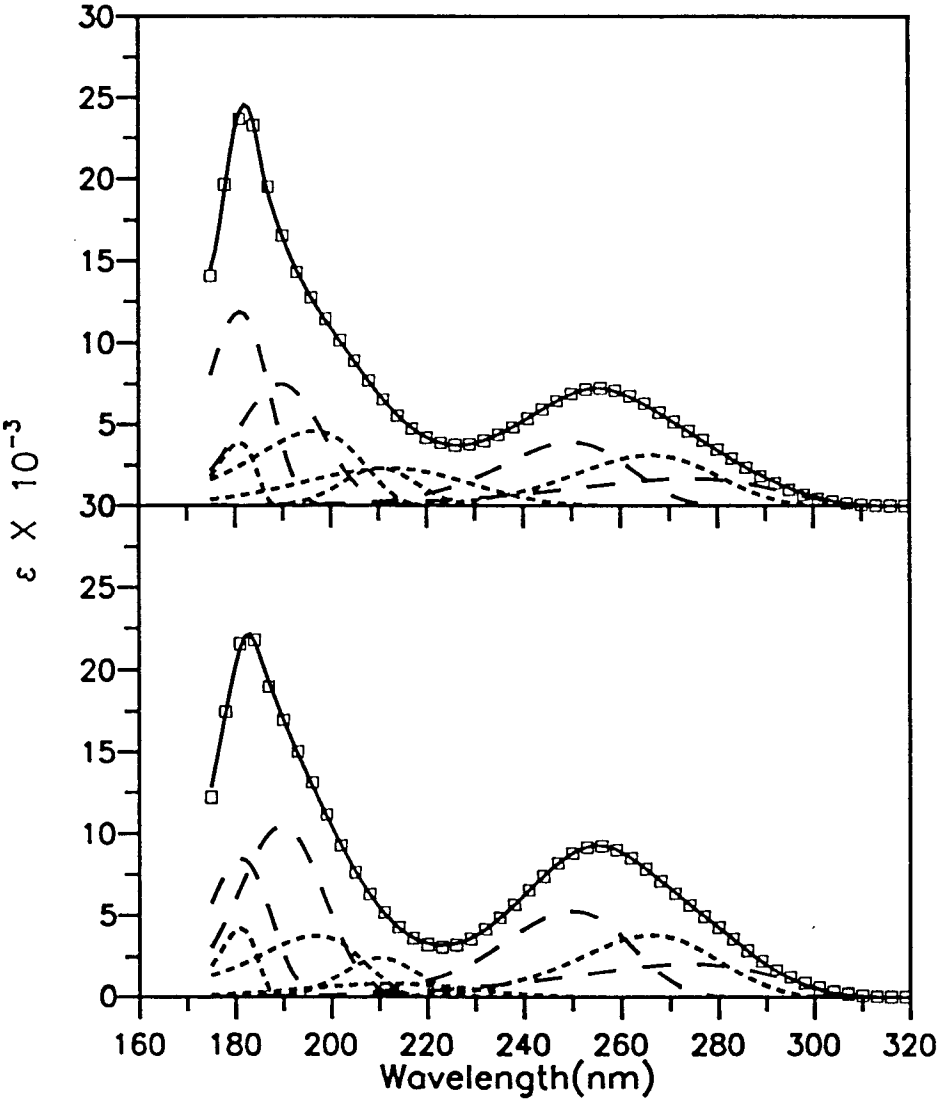


Figure III.2c

Figure III.3: Decomposition of the normal isotropic absorption (top) and flow linear dichroism (bottom) spectra for poly(dGdC)-poly(dGdC) in 10 mM sodium phosphate buffer, pH 7.0. The bands due to dG (— — —) and the bands due to dC (------) combine to give the fitted spectrum (––––) that is compared to the measured spectrum (□ □ □).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300
320
Wavelength(nm)

Figure III.3

Table III.1

Monomer absorption bands and transition dipole directions

base	λ(nm)[a]	ζ x 10^{-3}[b]	σ(nm)[c]	ρ[d]	δ (deg)[e]
guanine	274.5	288.7	16.7	1.50	-4
	248.5	309.5	13.9	1.10	-75
	198.8	471.2	11.6	1.03	-71
	183.2	449.4	11.6	1.50	41
cytosine	269.0	301.2	15.3	1.12	6
	228.1	319.2	19.8	1.31	-35
	211.6	86.8	7.1	1.00	76
	196.5	403.1	9.9	1.43	86
	170.1	94.0	12.4	1.03	0

[a]wavelength maximum of decomposed bands.
[b]intensity of decomposed bands (in units of $nm \cdot L \cdot mol^{-1} cm^{-1}$).
[c]half the bandwidth at half height.
[d]skewness factor.
[e]direction of transition dipole (refs. 37-38).

Table III.2

Absorption bands, inclination angles (α), and axes of inclination (χ) for alternating copolymers[a]

Base	μ(nm)	$\zeta \times 10^{-3}$	σ(nm)	ρ	α(deg)	χ(deg)
Poly(dGdC)-poly(dGdC) in buffer						
dG	274.2 ± 0.9	91.9 ± 2.6	25.1 ± 0.5	1.50 ± 0.00	25.5 ± 1.1	136.6 ± 3.8
	249.9 ± 0.4	132.5 ± 2.2	15.7 ± 0.2	1.28 ± 0.05	(21.4 ± 0.5)	(130.7 ± 2.8)
	189.7 ± 0.6	166.8 ± 4.7	10.5 ± 0.4	1.08 ± 0.06		
	181.1 ± 0.1	195.6 ± 3.3	7.7 ± 0.3	1.18 ± 0.05		
dC	266.2 ± 0.5	115.3 ± 3.3	17.3 ± 0.6	1.20 ± 0.03	32.5 ± 1.2	217.6 ± 2.8
	212.3 ± 1.2	101.6 ± 8.5	20.8 ± 1.1	1.14 ± 0.08	(34.0 ± 0.7)	(184.0 ± 3.2)
	210.1 ± 0.7	44.4 ± 2.5	9.1 ± 0.7	1.02 ± 0.07		
	196.7 ± 0.5	140.1 ± 6.8	13.9 ± 0.4	1.50 ± 0.00		
	181.0 ± 0.3	40.7 ± 9.0	4.8 ± 0.4	1.50 ± 0.00		
Poly(rGrC)-poly(rGrC) in buffer						
rG	269.3 ± 0.0	93.9 ± 0.5	20.3 ± 0.1	1.20 ± 0.00	29.8 ± 0.3	92.4 ± 0.5
	255.9 ± 0.1	96.6 ± 0.4	17.8 ± 0.0	1.42 ± 0.01		
	199.3 ± 0.1	170.0 ± 0.9	13.6 ± 0.1	1.04 ± 0.01		
	183.0 ± 0.0	174.4 ± 0.6	7.8 ± 0.0	1.30 ± 0.00		
rC	264.1 ± 0.0	81.2 ± 0.2	16.7 ± 0.0	1.01 ± 0.00	36.8 ± 0.2	67.8 ± 0.8
	235.5 ± 0.1	95.9 ± 0.3	20.9 ± 0.0	1.50 ± 0.00		
	211.5 ± 0.1	34.2 ± 0.3	6.7 ± 0.0	1.12 ± 0.02		
	195.7 ± 0.1	117.7 ± 0.5	11.8 ± 0.0	1.38 ± 0.02		
	176.0 ± 0.0	84.2 ± 0.1	6.9 ± 0.0	1.49 ± 0.01		

(Table III.2, continued)

Poly(dGdC)-poly(dGdC) in 80% TFE						
dG	279.4 ± 0.4	93.7 ± 2.7	20.0 ± 0.6	1.60 ± 0.06	30.6 ± 1.1	-36.7 ± 3.1
	249.3 ± 0.4	131.4 ± 2.8	17.3 ± 0.5	1.00 ± 0.01		
	195.3 ± 0.9	208.6 ± 8.8	13.5 ± 0.5	1.26 ± 0.06		
	170.4 ± 0.1	239.4 ± 5.3	8.2 ± 0.4	1.00 ± 0.09		
dC	261.9 ± 0.5	80.7 ± 4.1	14.9 ± 0.5	1.34 ± 0.04	35.0 ± 1.8	24.0 ± 3.8
	215.9 ± 0.9	146.8 ± 5.8	20.0 ± 0.7	1.54 ± 0.13		
	207.6 ± 0.7	46.7 ± 4.1	7.7 ± 0.7	1.00 ± 0.04		
	186.5 ± 0.6	180.7 ± 6.2	11.8 ± 0.6	1.35 ± 0.08		
	181.6 ± 0.2	110.7 ± 9.0	5.8 ± 0.1	1.00 ± 0.01		

[a]Symbols as in Table III.1.

sodium phosphate buffer, pH 7.0, is shown in Figure III.1. This RNA has a CD characteristic of the A-form with a positive band at 266 nm, a negative band at 208 nm, and an intense positive band at 188 nm, in agreement with the early report [30].

The normalized A, LD, and L' spectra of poly(rGrC)-poly(rGrC) are new and shown in Figure III.2b. The shape of the L' spectrum for the A-form RNA polymer is quite varied, and different from that of B-form DNA polymer in buffer. The decomposition and interchange base inclination data for the A-form RNA polymer resulting from the analysis of A and LD simultaneously are given in Table III.2. The measured α angles of 31.6° for rG and 35.2° for rC in A-form poly(rGrC)-poly(rGrC) are larger than the B-form values for poly(dGdC)-poly(dGdC), as expected.

We are interested in the base inclination changes in DNA molecules caused by the transition from B-form to A-form, which can be induced by dehydrating conditions. TFE is commonly used as the dehydrating solvent, and for polymers with a high GC content, the B- to A-form transformation is easier than with a high AT content [39]. Also, the alternating DNA polymers usually give a better A-form transition than the homo-duplex polymers under same conditions [39,40]. Many previous spectroscopic results have shown that 80% TFE and a low salt buffer cause a complete B to A transition for most DNA polymers [31,40].

The CD of poly(dGdC)-poly(dGdC) in 80% TFE, 0.67 mM sodium phosphate buffer, pH 7.0, is shown in Figure III.1. It is quite similar to the CD of poly(rGrC)-poly(rGrC), confirming the transition to the A-form under dehydrating conditions. However, the band intensities

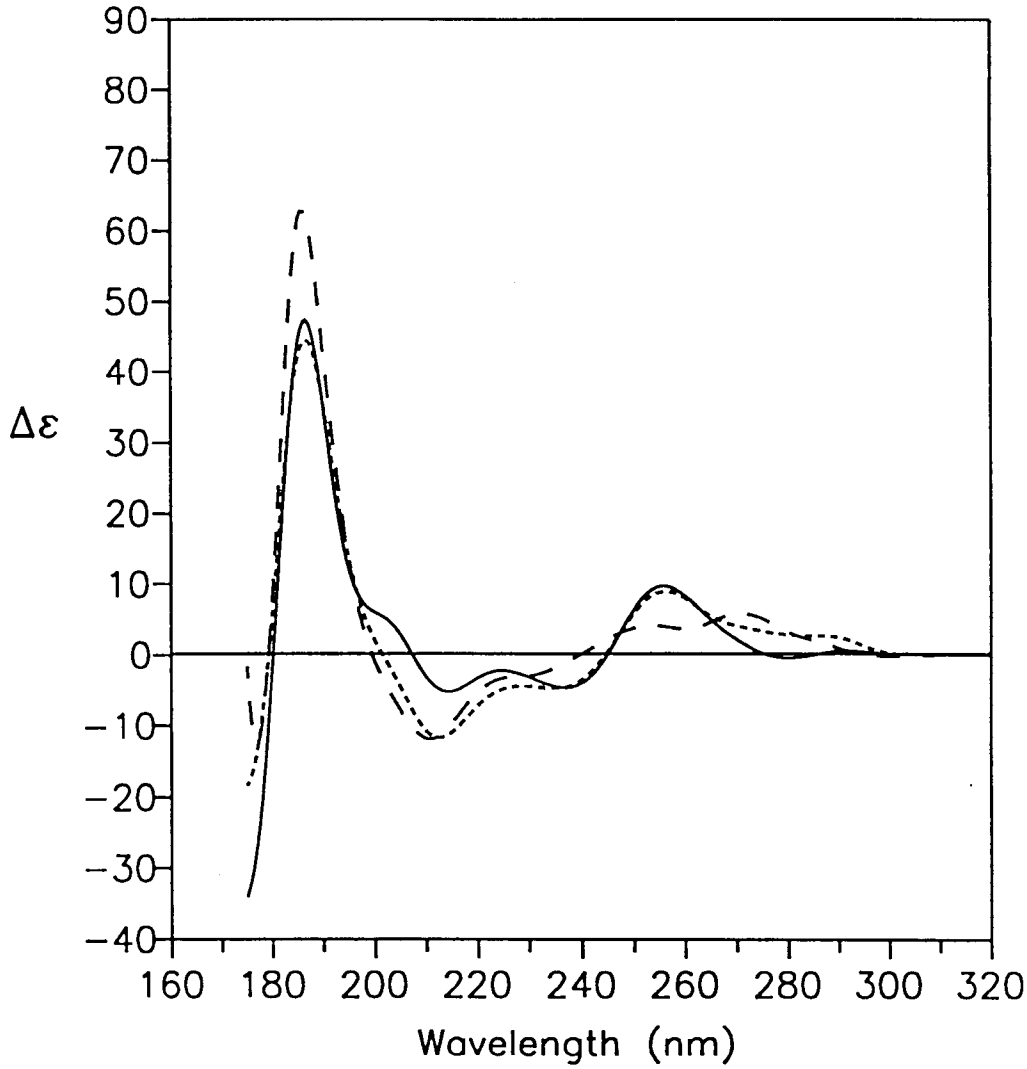
for the RNA are slightly smaller than the corresponding bands of A-form DNA, and the positions also have slight shifts. The positive band at short wavelength is higher in intensity and shifted to slightly shorter wavelengths compared to the CD of B-conformation, but the CD has the typical negative A-form band at about 210 nm.

The A ,LD, and L' spectra of poly(dGdC)-poly(dGdC) in 80% TFE, which are shown in Figure III.2c, are new, and are analyzed for base inclinations. There are no large differences between the spectra of normalized A and LD in 80% TFE for the A-form and in buffer for the B-form. However, the L' spectrum has stronger variation and the peak shifts to 220 nm. The results from decomposition of the A spectrum and simultaneous fitting of the LD spectrum are listed in Table III.2. The inclination angles of 30.6° for dG and 35.0° for dC are both larger than the B-form, and similar to the RNA angles, but the axes are quite different from the RNA. This means that even though the LD for the B- and the A-forms of the DNA do not show large differences, the conformational changes in the TFE solvent causing the larger base inclinations in the A-form can still be detected.

Poly(dG)-poly(dC) and poly(rG)-poly(rC)

The base inclinations in buffer for two homo-duplex polymers, poly(dG)-poly(dC) and poly(rG)-poly(rC), are compared to poly(dG)-poly(dC) under dehydrating conditions. The CD spectrum of B-form poly(dG)-poly(dC) in 10 mM sodium phosphate buffer, pH 7.0, is shown in Figure III.4. This CD has positive band at 258 nm, negative

Figure III.4: The circular dichroism of poly(dG)-poly(dC) in 10 mM sodium phosphate buffer, pH 7.0 (----), poly(dG)-poly(dC) in 80% 2,2,2-trifluoroethanol, 0.67 mM sodium phosphate buffer, pH 7.0 (------), and poly(rG)-poly(rC) in 10 mM sodium phosphate buffer (— — —).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30
20
10
0
−10
−20
−30
−40
Δε
160
180
200
220
240
260
280
300
320
Wavelength (nm)

Figure III.4

bands at 238 and 213 nm, and an intense positive band at 188 nm, in agreement with the previous report [27]. The A, LD and L' spectra measured from 320 nm to 175 nm in the same buffer are new, and all the normalized spectra are plotted in Figure III.5a. The L' spectrum for B-form poly(dG)-poly(dC) is quite varied and has a peak at 224 nm. This variation of the L' spectrum with wavelength demonstrates that the bases in B-form poly(dG)-poly(dC) are inclined with respect to the helix axis. The decomposition data, inclination angles and axes of inclination for the bases in B-form poly(dG)-poly(dC) are given in the Table III.3. The α angles of 21.5° for dG and 34.2° for dC are quite close to the results calculated earlier [27,24], which are listed in parentheses in Table III.3. These B-form inclination angles are smaller than for the alternating GC polymer, but still larger than GC bases in natural B-form DNA [24].

The CD of the ribo-homo polymer poly(rG)-poly(rC) in 10 mM sodium phosphate buffer is also shown in Figure III.4. The CD for this RNA has positive bands around 260 nm, a negative band at 210 nm, and a very intense positive band at 188 nm, typical of the A-form [28]. The A, LD, and L' spectra shown in Figure III.5b are new. The shape of the L' spectrum is less varied than for B-form poly(dG)-poly(dC), and the base inclination angles of 23.0° for rG and 33.0° for rC (see Table III.3) are not much different.

The CD spectrum of homo-duplex poly(dG)-poly(dC) in 80% TFE, 0.67 mM sodium phosphate, pH 7.0, shown in Figure III.4, is intermediate between the CD of the B-form DNA and the A-form RNA. It does have a deeper negative band shifted to 212 nm, which is characteristic of the A-form. The normalized A, LD and L' spectra

Figure III.5a. The normalized A in absorbance units (-----), normalized LD with sign reversed (— — —), and normalized L' (- - - - -) spectra for poly(dG)-poly(dC) in 10 mM sodium phosphate buffer, pH 7.0.

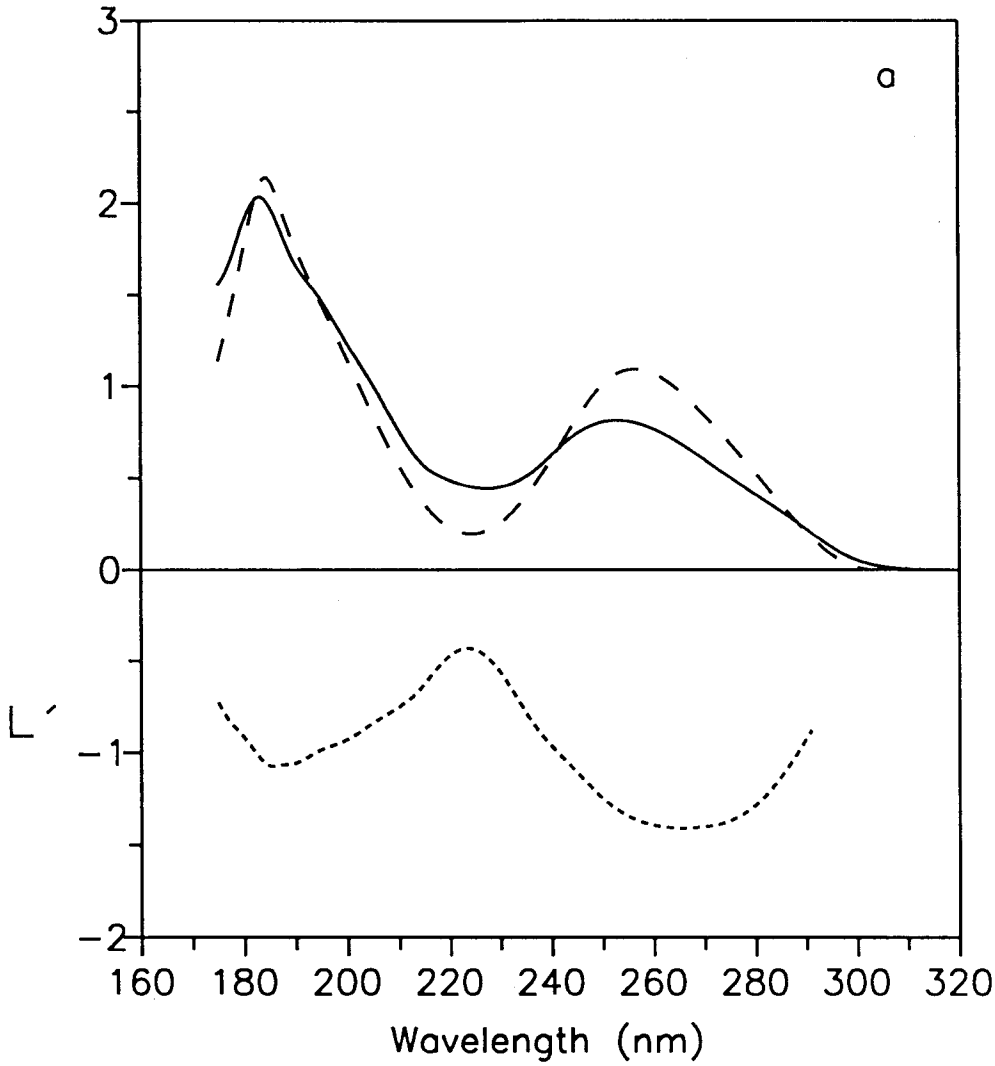

Figure III.5a

Figure III.5b. The normalized A in a absorbance units (-----), normalized LD with sign reversed (— — —), and normalized L' (- - - - -) spectra for poly(rG)-poly(rC) in 10 mM sodium phosphate buffer.

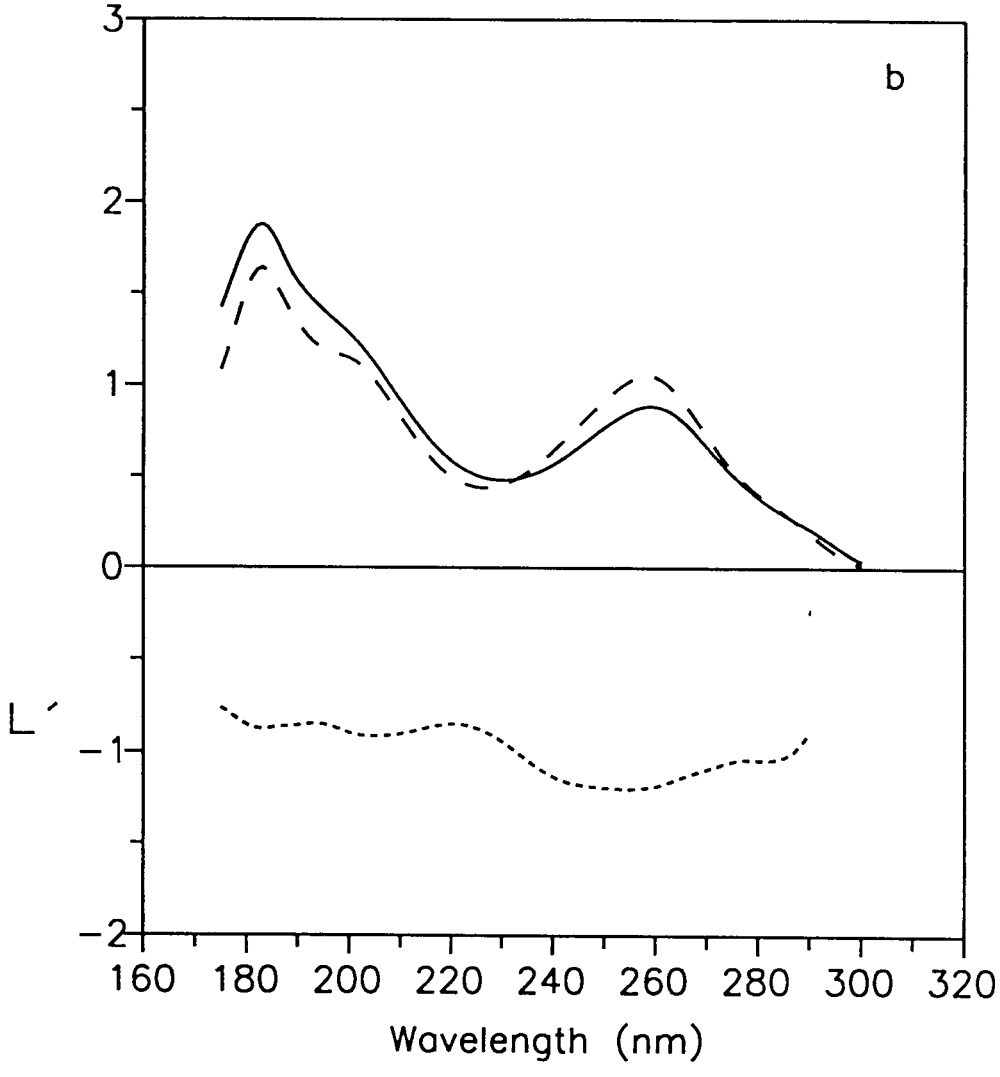

Figure III.5b

Figure III.5c. The normalized A in a absorbance units (——), normalized LD with sigh reversed (— — —), and normalized L' (- - - - -) spectra for poly(dG)-poly(dC) in 80% TFE, 0.67 mM sodium phosphate buffer, pH 7.0.

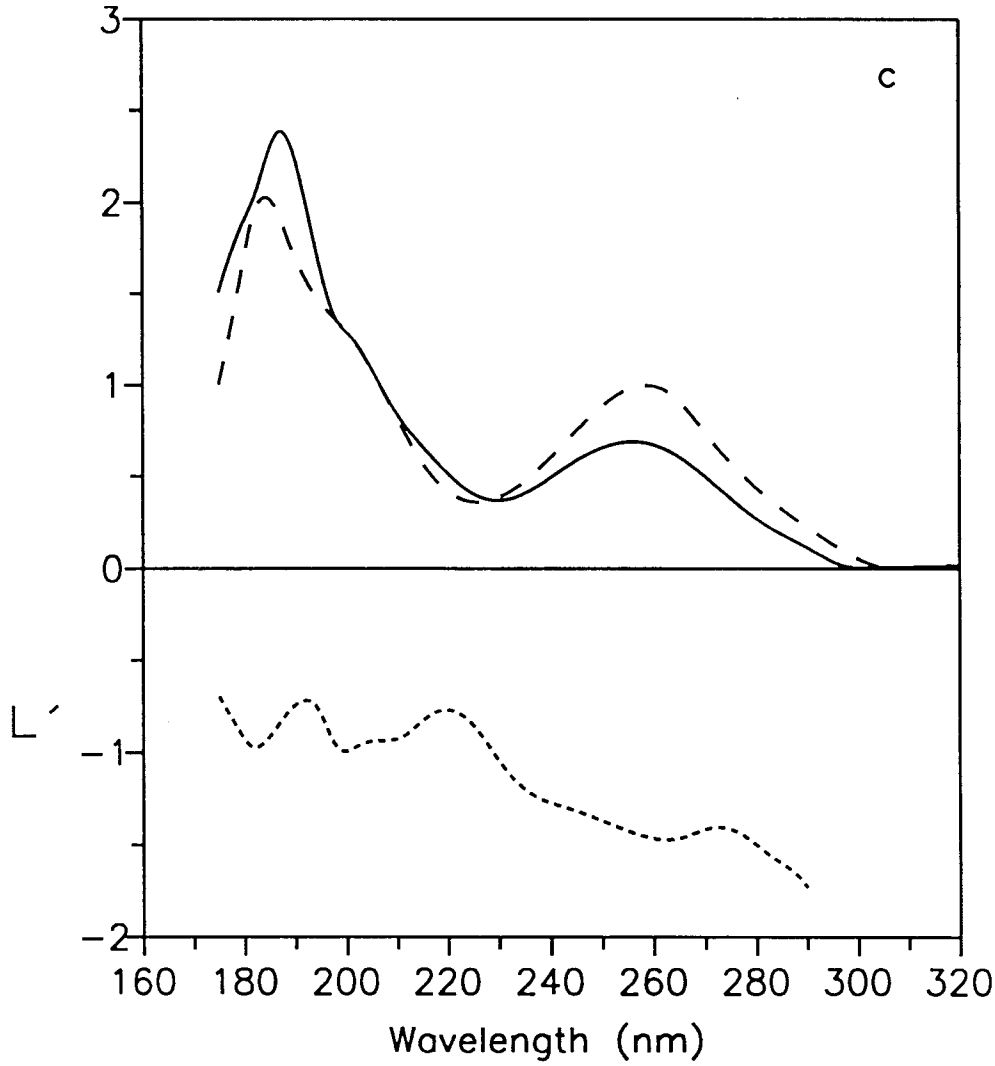

Figure III.5c

Table III.3

Absorption bands, inclination angles (α), and axes of inclination (χ) for homopolymers[a]

Base	μ(nm)	ζ x 10^{-3}	σ(nm)	ρ	α(deg)	χ(deg)
Poly(dG)-poly(dC) in buffer						
dG	277.3 ± 0.1	85.7 ± 0.9	19.9 ± 0.2	1.50 ± 0.00	22.4 ± 0.2	114.3 ± 1.5
	251.3 ± 0.1	103.1 ± 0.8	13.1 ± 0.1	1.00 ± 0.01	(20.1 ± 0.6)	(116.8 ± 3.5)
	195.4 ± 0.1	168.8 ± 0.8	12.1 ± 0.0	1.02 ± 0.01		
	179.7 ± 0.0	191.1 ± 0.4	9.2 ± 0.0	1.26 ± 0.00		
dC	267.0 ± 0.1	94.7 ± 1.0	15.1 ± 0.1	1.05 ± 0.01	38.5 ± 0.2	199.8 ± 0.8
	231.2 ± 0.2	138.5 ± 0.6	22.1 ± 0.1	1.34 ± 0.01	(33.8 ± 1.0)	(189.8 ± 3.8)
	214.2 ± 0.2	34.8 ± 0.8	8.8 ± 0.3	1.04 ± 0.01		
	196.5 ± 0.2	157.5 ± 0.7	18.0 ± 0.1	1.50 ± 0.00		
	184.9 ± 0.0	64.0 ± 0.6	8.1 ± 0.0	1.03 ± 0.01		
Poly(rG)-poly(rC) in buffer						
rG	276.9 ± 0.1	102.9 ± 0.7	20.3 ± 0.1	1.50 ± 0.00	23.0 ± 0.5	111.5 ± 2.7
	253.5 ± 0.1	121.6 ± 0.4	16.0 ± 0.1	1.45 ± 0.01		
	198.5 ± 0.2	102.6 ± 0.7	13.4 ± 0.1	1.01 ± 0.01		
	177.2 ± 0.1	171.4 ± 0.3	10.8 ± 0.0	1.00 ± 0.00		
rC	262.3 ± 0.1	97.5 ± 0.5	16.1 ± 0.1	1.12 ± 0.01	33.0 ± 0.7	192.5 ± 2.7
	221.0 ± 0.1	106.0 ± 0.4	20.5 ± 0.2	1.42 ± 0.01		
	211.6 ± 0.4	28.1 ± 1.0	8.6 ± 0.1	1.01 ± 0.01		
	192.0 ± 0.2	127.6 ± 0.9	14.9 ± 0.2	1.50 ± 0.00		
	182.5 ± 0.0	50.5 ± 0.4	6.2 ± 0.1	1.00 ± 0.01		

(Table III.3, continued)

Poly(dG)-poly(dC) in 80% TFE

dG	279.8 ± 0.5	66.8 ± 1.3	23.7 ± 0.2	1.50 ± 0.00	30.8 ± 0.3	154.5 ± 0.3
	252.7 ± 0.1	105.5 ± 1.1	15.4 ± 0.1	1.09 ± 0.01		
	197.8 ± 0.1	185.0 ± 0.9	12.0 ± 0.1	1.06 ± 0.01		
	186.9 ± 0.1	208.0 ± 0.7	12.1 ± 0.1	1.50 ± 0.00		
dC	261.3 ± 0.1	77.8 ± 1.0	15.7 ± 0.1	1.02 ± 0.01	36.8 ± 0.3	193.8 ± 0.8
	220.4 ± 0.3	120.6 ± 1.6	27.0 ± 0.3	1.50 ± 0.00		
	210.3 ± 0.3	68.9 ± 0.5	12.8 ± 0.2	1.10 ± 0.01		
	189.8 ± 0.3	138.5 ± 0.6	12.8 ± 0.1	1.50 ± 0.00		
	182.4 ± 0.0	70.1 ± 0.6	6.9 ± 0.0	1.33 ± 0.02		

[a]Symbols as in Table III.1.

are all new and shown in Figure III.5c. The L' spectrum is similar to that for the RNA, but more varied. The base inclination angles and other decomposition data are listed in Table III.3. Poly(dG)-poly(dC) in 80% TFE has slightly larger inclination angles of 23.6° for dG and 35.2° for dC, similar to the RNA, but the axes are similar to the B-form DNA. Therefore, although the CD for poly(dG)-poly(dC) in 80% TFE is different from the B-form in buffer, the base orientation is still B-form, in analogy with the results for poly(dA)-poly(dT) in the accompanying paper. In general changes in base inclination between the homo-duplex DNA polymer in buffer and in 80% TFE, and even base inclinations for the RNA homo-polymer in buffer, are smaller than the differences occurring between the A- and B- forms for the alternating polymers.

The similarity in spectra and structure for poly(dG)-poly(dC) in 80% TFE and poly(rG)-poly(rC) in buffer proves that the dehydrating conditions puts the DNA into the A-form. However, base inclination differs little between the A- and B-forms. Changes in base inclination between the DNA in buffer and in 80% TFE, and even base inclinations for the RNA polymer in buffer, are smaller than the differences occurring between the A- and B-forms for the alternating polymers.

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Section IV

Conclusions

The secondary structure of nucleic acids in solution is affected by environmental conditions, such as solvent, pH, temperature, and ionic strength. The base inclinations of nucleic acids are among the important parameters that define the secondary structures. In this work, we focus on the base inclinations of synthetic ribo- and deoxyribo-polymers in 10 mM phosphate buffer (pH 7.0) and under dehydrating conditions, such as 80% TFE. The circular dichroism, absorption, and linear dichroism spectra are investigated and analyzed for inclination angles and axes of the DNA polymers and RNA polymers in different solvents. The results and discussion in Sections II and III comes to the following conclusions:

1. Secondary structures of all the synthetic polynucleotides studied in this work can be different depending on the solution conditions. The DNA polymers are usually in the B-form in 10 mM sodium phosphate buffer, pH 7.0; while synthetic RNA polymers in the same buffer are in the A-form. These results are confirmed by CD spectra and in agreement with earlier reports.

2. Synthetic alternating DNA polymers undergo a B-form to A-form transition in 80% TFE; while the homo-duplex DNA polymers can not go into the A-form under the same conditions. The homopolymer poly(dA)-poly(dT) has many unusual and distinct structural features that differentiate it from other B-type DNA sequences. As mentioned in previous studies, the helical repeat of

poly(dA)-poly(dT) in solution was found to be 10.0 base pairs per turn, in contrast to 10.5 base pairs per turn for the random DNA and alternating polymers. The axial rise of this homopolymer is also less than that found for other DNA fibers. X-ray analysis of the crystal structure for oligo (dA)-(dT) showed that a large propeller twist existed at each A-T base pair, resulting in the formation of some non-Watson-Crick cross-strand hydrogen bonds. Therefore, these unusual structural features cause the special structural behaviors for the homopolymer poly(dA)-poly(dT) in solution.

The results from our studies on structural transitions of homopolymers in solution indicate that the conformation of poly(dA)-poly(dT) in 80% TFE remains in the B-form; poly(dG)-poly(dC) in 80% TFE is also quite similar to the B-form, although the CD spectrum shows that a partial transition may occur. However, the structure of poly(dA)-poly(dT) in TFE solvent is really in a dynamic transition, which can be detected through CD measurements (data not shown here). This may provide the possibility of B- to A-form transition of this homopolymer under special environmental conditions.

3. Bases in all the synthetic DNA polymers and RNA polymers studied here are inclined with respect to the helix axis. Usually, the inclinations for A-form polymers are larger than for the B-form polymers; the alternating polymers give larger inclinations than the corresponding homo-duplex polymers. Also, the inclinations of purine bases are more sensitive to the solution conditions than that of pyrimidine bases. Our results show that A-form alternating AT and GC polymers give larger inclinations than their corresponding

polymers in buffer, especially the bases dA and dG (see Table III.2 in both section II and III). The homopolymers poly(dA)-poly(dT) and poly(dG)-poly(dC) are more stable to changes in solution, which can be seen as slight changes in their inclination angles (see Table III.3 in sections II and III).

4. Changes in solution from aqueous buffer to dehydrating solvent indeed affect the secondary structure of nucleic acids, which can be detected by using circular dichroism. Linear dichroism measures the base inclinations which vary with the changes in environmental conditions. In our studies, all the information from spectral measurements and analysis is critical and important. With the help of more sensitive instruments and a designed computational data analysis program, we investigate deeply the dramatic structural changes of polynucleotide molecules in different solutions. Many previous reports in this field focused on the behaviors of molecules in crystal and fibers, which limited the significant connections between structure and function, since most real biological reactions happen in solution. Our studies focus on measuring and discussing the structural transitions of selected DNA and RNA polymers in aqueous solution and under dehydrating conditions, which is significant for a better understanding of the functions of synthetic polymers in real biological systems.

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