Z-DNA: Vacuum ultraviolet circular dichroism

(DNA structure/left-handed double helix/differential scattering of circularly polarized light/synchrotron radiation)

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ABSTRACT In concentrated salt or ethanolic solutions, the self-complementary copolymer poly(dG-dC)·poly(dG-dC) forms a left-handed double-helical structure that has been termed “Z-DNA.” The first evidence for this structure came from changes observed in the circular dichroism (CD) spectrum between 230 and 300 nm for low- and high-salt solutions (Pohl, F. M. & Jovin, T. M. (1972) *J. Mol. Biol.* 67, 675–696). In 3 M NaCl, the CD spectrum is approximately inverted compared to the B-form spectrum observed in low-salt solution. We measured the vacuum ultraviolet CD spectrum of poly(dG-dC)·poly(dG-dC) down to 180 nm under conditions in which the 230- to 300-nm spectrum is inverted. Below 200 nm, where the B form exhibits the large positive peak at 187 nm that is characteristic of right-handed double-helical DNAs, the Z form exhibits a large negative peak at 194 nm and a positive band below 186 nm. Therefore, the Z-form vacuum ultraviolet CD spectrum resembles an inverted and red-shifted B-form spectrum. The magnitudes of the differences observed between the B and Z forms in the CD spectrum below 200 nm are about 10 times greater than those observed between 230 and 300 nm. The vacuum ultraviolet CD spectrum of poly(dG-dC)·poly(dG-dC) in 3 M Cs₂SO₄ also is inverted compared to the B-form spectrum; however, between 230 and 300 nm, it is nonconservative with a negative maximum at 290 nm and a weak positive CD signal above 300 nm, presumably reflecting differential light scattering and indicating the existence of molecular aggregates. Our results suggest that the vacuum ultraviolet CD spectrum is sensitive to the handedness of double-helical DNA structures. The CD spectrum in this region should complement other spectroscopic methods in relating the structures of poly(dG-dC)·poly(dG-dC) existing in solution to those determined in the solid state by x-ray crystallography.

The first indications of the existence of the Z form of DNA were the dramatic changes in the 230- to 300-nm circular dichroism (CD) spectrum of the copolymer poly(dG-dC)·poly(dG-dC) produced by increasing salt concentrations (1). In low concentrations of NaCl, the 230- to 300-nm CD spectrum is approximately conservative, with a positive peak near 275 nm and a negative peak near 253 nm, resembling the spectrum of B-form DNA. As the concentration of NaCl is increased, a cooperative transition with a midpoint at about 2.5 M NaCl leads to an inverted CD spectrum with a negative maximum near 292 nm and a positive maximum near 265 nm.

The dramatic spectral changes caused by high salt (1–5) prompted several x-ray diffraction investigations (6–8) of the three-dimensional structure of crystals of short, defined lengths of dG-dC oligomers and led to the discovery of what is now called Z-DNA, a zig-zag, left-handed helix in which the guanine residues adopt a *syn* conformation relative to the glycosidic bond. Fiber diffraction patterns of much longer chains of

poly(dG-dC)·poly(dG-dC) have been interpreted as having a similar conformation (9). Crystallographic studies show that more than one form of the left-handed Z-type structure can exist in the solid state (7, 8).

The physiological significance of Z-DNA is conjectural at present, but there are indications that damage to DNA by chemical mutagens and carcinogens, particularly formation of adducts at the 8-position of purines, tends to favor the *syn* conformation (10) and, hence, induce a local Z-like structure (11–13). There are also indications of repair nucleases directed towards double-stranded DNA containing purines modified at the 8-position and, hence, presumably in the *syn* conformation (14).

Several laboratories have extended measurements of the CD of DNA into the vacuum ultraviolet region—i.e., wavelengths less than about 200 nm (15–18). Lewis and Johnson (17) showed that heat denaturation of duplex DNA results in much larger decreases in the magnitude of the CD effect for wavelengths less than 200 nm than for longer wavelengths. In contrast, the CD at 230–300 nm appears to be more indicative of the type of right-handed helical structure (i.e., A, B, or C) than is the CD in the region below 200 nm (18). Above 230 nm, the CD spectrum of B-form DNA is semiconservative with a positive maximum near 270 nm and a negative maximum near 250 nm, whereas the A and C forms have nonconservative spectra, with the dominant features being a positive maximum above 260 nm for the A form and a negative maximum below 260 nm for the C form (18, 19). Below 200 nm, the general shape and peak positions of the CD spectra of the A, B, and C forms are similar. The CD of the B and C forms are almost identical, whereas the CD of the A form differs mainly in having a greater magnitude (18).

To determine if the vacuum ultraviolet CD spectrum might be sensitive to the handedness of the DNA helix, we measured the vacuum ultraviolet CD spectrum of poly(dG-dC)·poly(dG-dC) under conditions that invert the 220- to 300-nm CD spectrum and, presumably, induce the left-handed Z conformation. We find major differences between the vacuum ultraviolet CD spectra of the B and Z forms; the magnitude of this difference is about 10 times greater below 200 nm than in the 230- to 300-nm region. This result suggests that the vacuum ultraviolet CD spectrum is an indicator of the handedness of the helicity of duplex DNA. Our results also should be useful in correlating the various Z-type structures determined in the solid state with the forms obtained in solution and may lead to a better understanding of the electronic transitions responsible for the spectrum of DNA below 200 nm.

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Abbreviation: CD, circular dichroism.

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This report demonstrates application of a synchrotron radiation source to obtain the circular dichroism characteristics of a biopolymer.

MATERIALS AND METHODS

Poly(dG-dC)·poly(dG-dC), lot no. 658-5, was purchased from P-L Biochemicals. Cesium sulfate (optical grade) was from Schwarz BioResearch (Orangeburg, NY). Sodium chloride was reagent grade. A concentrated poly(dG-dC)·poly(dG-dC) sample in phosphate buffer was prepared, and equal aliquots were added to salt solutions so that, for a given salt, all samples contained the same concentrations of poly(dG-dC)·poly(dG-dC) in 10 mM potassium phosphate buffer (pH 7.0). The salt concentrations were either 0.2 M or 3.0 M in NaCl or Cs₂SO₄. Reference solutions containing the same concentrations of buffer and salt were prepared for each sample. Similar samples were also prepared in 50% or 70% trifluoroethanol (Aldrich; gold label grade). These solutions were 10 mM in phosphate buffer but contained no additional salt.

Absorption spectra were recorded with a Cary 118C spectrophotometer (Varian, Palo Alto, CA) operated under computer control (20). CD spectra were recorded with the Synchrotron Ultraviolet Project spectrometer operating at the National Bureau of Standards synchrotron storage ring (Gaithersburg, MD). A preliminary description of the spectrometer has appeared elsewhere (21). Recent improvements include fitting the monochromator with a blazed holographic diffraction grating (Bausch and Lomb, Rochester, NY), which significantly reduces the intensity of stray radiation at the sample. CD spectra for wavelengths greater than 230 nm were checked with a conventional-source spectrometer (22). Samples were placed in cylindrical quartz cuvettes with an optical path length of 1 mm, or in a variable pathlength cell with either Suprasil quartz or calcium fluoride windows. The variable pathlength cell was modified for operation in a vacuum. The concentration of polymer and optical pathlength were chosen so that the total maximum absorbance of the solution was less than 1.5.

RESULTS AND DISCUSSION

The circular dichroism spectra of poly(dG-dC)·poly(dG-dC) in low (0.2 M) and high (3 M) NaCl are shown in Fig. 1. From 230 to 300 nm, our CD spectra are similar to those reported by Pohl and Jovin (1). The high-salt spectrum is approximately inverted compared to the spectrum taken in low-salt solutions. The low-salt 230- to 300-nm CD spectrum is similar to the semiconservative spectrum observed for B-type DNAs.

The spectra reported by Pohl and Jovin extend only to about 230 nm. Below 220 nm, the approximate mirror symmetry between the B- and Z-type spectra ends. Both spectra are negative and similar in magnitude down to about 210 nm. The low-salt CD spectrum has a negative maximum at about 208 nm and goes positive at about 201 nm. This is the expected behavior for right-handed double-helical nucleic acids because all of the DNAs that have been studied exhibit an intense positive CD maximum between 185 and 190 nm (15–19). In contrast, the spectrum of poly(dG-dC)·poly(dG-dC) in 3 M NaCl becomes increasingly negative down to 205 nm, the limit imposed on the high-salt spectrum by absorption of NaCl.

The CD spectrum below 205 nm is particularly important because Johnson and his colleagues (17, 18) have reported that the magnitude of the 188-nm band is more sensitive to base composition and more severely perturbed by thermal denaturation than is the 230- to 300-nm spectrum. Thus, we wished to investigate a system that both produces a 230- to 300-nm CD spectrum similar to that induced by 3 M NaCl and is transparent

FIG. 1. Circular dichroism spectra of poly(dG-dC)·poly(dG-dC) (0.42 mM in nucleotides) in 0.2 M (----) and 3 M NaCl (—). Both spectra for wavelengths greater than 230 nm are similar to those reported by Pohl and Jovin (1) and demonstrate the inversion of the CD spectrum induced by high concentrations of NaCl. These spectra were not extended below 200 and 205 nm, respectively, because of the opacity of NaCl. Circular dichroism is plotted as the difference between the molar extinction coefficients for left and right ($\epsilon_L - \epsilon_R$) circularly polarized light; the units are $M^{-1}cm^{-1}$.

below 205 nm. Other solutes that have been reported to induce the CD (or corresponding optical rotatory dispersion) spectrum typical of Z-DNA include NaClO₄, MgCl₂ (1), and 60% (vol/vol) ethanol (4). Whereas these compounds are not particularly well suited for vacuum ultraviolet spectroscopy, fluorinated alcohols are (23). Thus, we investigated the effect of trifluoroethanol with the results shown in Fig. 2. For solutions up to 60% (vol/vol) in trifluoroethanol, the 230- to 300-nm CD spectrum of poly(dG-dC)·poly(dG-dC) is similar to the low-salt spectrum characteristic of B-form DNA. In the vacuum ultraviolet region, a large positive maximum is observed at about 187 nm—the result expected for a right-handed double helix.

Increasing the concentration of trifluoroethanol to 70% drastically changes the CD spectrum in both the 230- to 300-nm region and in the vacuum ultraviolet region. In the 230- to 300-nm region, the CD is similar to that induced by high concentrations of ethanol (4) and NaCl (ref. 1 and Fig. 1) and is presumably indicative of a left-handed double-helical structure. At around 210 nm the CD spectrum of the 70% trifluoroethanol

FIG. 2. Circular dichroism of a 0.035 mM solution of poly(dG-dC)·poly(dG-dC) in 50% (----) and 70% (—) trifluoroethanol. Above 230 nm, the spectra are similar to those reported by Pohl (4) and Pohl and Jovin (1) for ethanolic and sodium chloride solutions. Between 205 and 220 nm, the spectra are similar to those for 0.2 M and 3 M NaCl (see Fig. 1). For wavelengths less than 200 nm, the spectrum for 70% trifluoroethanol is inverted and red-shifted compared to that for 50% trifluoroethanol. These spectra suggest that the vacuum ultraviolet CD spectrum of duplex DNA is sensitive to the handedness of the double helix. $\epsilon_L - \epsilon_R$, Difference between the molar extinction coefficients for left and right circularly polarized light.

sample is similar to that for the sample in 50% trifluoroethanol, a result that parallels the data for high and low concentrations of NaCl (see Fig. 1).

Below 205 nm, the CD spectrum of poly(dG-dC)·poly(dG-dC) in 70% trifluoroethanol shows a large, negative maximum at about 194 nm and a positive band below 185 nm. Pending assignment of the transitions responsible for the positive CD band at 187 nm in the 50% trifluoroethanol sample and the negative band at 194 nm in the 70% trifluoroethanol sample, the vacuum ultraviolet CD spectrum of the latter can be described as the red-shifted mirror image of the former. The spectra in Fig. 2 suggest that the vacuum ultraviolet CD spectrum is sensitive to the handedness of the double helix. The magnitude of the changes observed in the vacuum ultraviolet is about 10 times greater than that observed at longer wavelengths.

The vacuum ultraviolet spectrum shown in Fig. 2 also reveals an apparent anomaly for the right-handed double-helical form of poly(dG-dC)·poly(dG-dC) (i.e., the species in 50% trifluoroethanol). Sprecher *et al.* (18) reported the vacuum ultraviolet CD of a series of DNAs for environmental conditions designed to produce the A, B, and C types of right-handed double helices. The positive maximum observed between 185 and 190 nm was of nearly equal magnitude for the B and C forms of a particular DNA, but in each case the peak was larger for the A form of that DNA. We have plotted the magnitudes of the maximum molar extinction coefficients for the 185- to 190-nm band reported by Sprecher *et al.* as a function of the G + C content of the DNAs used. We find a linear relationship with a positive slope for both the A and B forms (data not shown). Extrapolating to 100% G + C gives a molar extinction difference of $83 \text{ M}^{-1}\text{cm}^{-1}$ for the A form and $60 \text{ M}^{-1}\text{cm}^{-1}$ for the B form. The corresponding value for poly(dG-dC)·poly(dG-dC) in 50% trifluoroethanol is clearly closer to that extrapolated from the A forms of DNA than from the B forms. The magnitude of the negative maximum near 210 nm is also similar to the corresponding feature of the A-form DNAs reported by Sprecher *et al.* (18) and qualitatively different from the spectra of B- and C-form DNAs at this wavelength. Thus, for wavelengths less than 230 nm, the CD spectrum of poly(dG-dC)·poly(dG-dC) in 50% trifluoroethanol seems to resemble the spectrum of DNA in the A configuration, although for longer wavelengths it resembles the spectrum of a B-type DNA.

While investigating concentrated salt solutions that transmit short-wavelength ultraviolet radiation better than do the chlorides, we measured the CD spectrum of poly(dG-dC)·poly(dG-dC) in cesium sulfate (Fig. 3). The 230- to 300-nm CD spectrum is altered drastically by 3 M Cs_2SO_4 , with a negative band at about 290 nm but without a corresponding positive band between 280 and 240 nm. There is also an apparent small positive signal for wavelengths greater than 310 nm, a region in which poly(dG-dC)·poly(dG-dC) does not absorb. The vacuum ultraviolet CD spectrum was extended to 193 nm for the 0.2 M Cs_2SO_4 solution and 198 nm for 3 M Cs_2SO_4 . In low-salt solutions, the CD spectrum is positive below 200 nm, whereas the spectrum in 3 M salt remains negative to 198 nm. Thus, the sign of the vacuum ultraviolet CD is opposite for spectra taken in low and high concentrations of Cs_2SO_4 , just as it is for spectra in trifluoroethanol.

Pohl (4) showed that the characteristic Z-type 230- to 300-nm CD spectrum, similar to that induced by NaCl, ethanol, and trifluoroethanol, could be induced by 4 M CsCl, whereas we have produced essentially the same Z-type CD spectrum with concentrated Na_2SO_4 (data not shown). These results suggest that the spectrum shown in Fig. 3 is not due exclusively to either the cesium cation or the sulfate anion, although we have not systematically explored the effects of varying the concentrations

FIG. 3. Circular dichroism of poly(dG-dC)·poly(dG-dC) in 0.2 M (---) and 3 M (—) Cs_2SO_4 . Spectra terminate at 193 and 198 nm, respectively, because of the opacity of the salt. In the range investigated, the vacuum ultraviolet CD spectrum is generally similar to that found for trifluoroethanol (see Fig. 2). The small positive signal recorded for wavelengths greater than 300 nm, a region in which poly(dG-dC)·poly(dG-dC) does not absorb, indicates an increase in light scattering suggestive of intermolecular aggregation. $\epsilon_L - \epsilon_R$, Difference between the molar extinction coefficients for left and right circularly polarized light.

of the polymer and of different anions and cations. The small positive CD signal observed above 300 nm in Fig. 3 is presumably due to differential scattering of right and left circularly polarized light and, hence, indicates the formation of aggregates of poly(dG-dC)·poly(dG-dC). Visual inspection of some of our samples of poly(dG-dC)·poly(dG-dC) in 3 M cesium sulfate revealed a gel-like appearance. The spectrum shown in Fig. 3 is in some ways reminiscent of some of the CD spectra of aggregates of DNA and various copolymers studied by Gray *et al.* (24) except that the sign of the long wavelength component is positive, whereas all of the spectra reported by Gray *et al.* show a negative CD scattering above 300 nm. Perhaps the sign of the CD signal due to differential scattering above 300 nm is also characteristic of the handedness of the double helix. The much greater magnitude of the CD spectrum below 200 nm appears to make it less sensitive to distortions induced by scattering than is the 230- to 300-nm spectrum.

The unusual conformations of poly(dG-dC)·poly(dG-dC) were first revealed by CD (1). Although CD and a variety of other spectroscopic measurements (3, 5) provided tantalizing glimpses of the nature of the unusual structure, it remained for crystallographic determinations at atomic resolution to bring the unique features of the structure into clear focus (6–8). These reports showed a left-handed double-helical structure with the guanosine residues in a *syn* conformation, giving rise to a zig-zag arrangement of the phosphate groups and leading to the name Z-DNA. However, more than one Z-type structure is possible (7, 8, 25). Thus, correlation of the structures determined in the solid state with those existing in solution is a process in which spectroscopy and, in particular, CD must play a prominent role. The spectral data obtained in the vacuum ultraviolet region will presumably complement those provided by the 230- to 300-nm region. The main obstacle to theoretical calculations relating helical structure to the vacuum ultraviolet CD spectrum is the lack of experimental information on the electronic transitions of the bases in this spectral region. Although Hug and Tinoco (26, 27) calculated sets of allowed and forbidden transitions that included the relevant energies, absorption spectroscopy cannot adequately resolve their spectra; perhaps this problem can be addressed by means of magnetic CD.

Our results suggest that the vacuum ultraviolet CD of nucleic acids is sensitive to the handedness of their helical structure and, thus, enhances the usefulness of this spectral region for

studies of the properties of double-helical nucleic acids in solution. The large increase in the magnitudes of the CD of the double helical forms below 200 nm compared to those at longer wavelengths will be useful for studying the effects on DNA structure of effector molecules with competing absorption and with CD effects that may mask the weaker CD of the DNA in the 230- to 300-nm region.

Although very little time has been available for development, the combination of source plus spectrometer we used to record the vacuum ultraviolet CD spectrum of Z-DNA has a sensitivity of about 10^{-5} absorbance units below 200 nm. This is similar to the sensitivity of current versions of the vacuum CD spectrometers of the type originally constructed by Johnson (28) and Schnepf *et al.* (29). The performance of our instrument would be expected to improve with continued development using the same light source. However, the spectrometer will soon be moved to the National Synchrotron Light Source, which is nearing completion at Brookhaven, where we expect a 50- to 100-fold increase in light intensity. A CD spectrometer also will be available at the Aladdin synchrotron radiation source being built in Wisconsin. We expect that the availability of high-performance spectrometers with synchrotron radiation sources will improve significantly the sensitivity and temporal resolution of circular dichroism measurements in the far and vacuum ultraviolet.

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